Downstream process design for microalgae

Sindri Freyr Ólafsson

Faculty of Industrial Engineering,
Mechanical Engineering and
Computer Science
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Sindri Freyr Ólafsson

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Magister Scientiarum degree in industrial engineering

Advisors
Sigurjón Arason
Sigurður Brynjólfsson
Björn Margeirsson
Hörður G. Kristinsson

External examiner
Sveinn Víkingur Árnason

Faculty of Industrial Engineering, Mechanical Engineering and Computer Science
School of Engineering and Natural Sciences
University of Iceland
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Faculty of Industrial Engineering, Mechanical Engineering and Computer Science
School of Engineering and Natural Sciences
University of Iceland
Hjarðarhaga 2-6
107, Reykjavik
Iceland

Telephone: 525 4000

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Abstract

Various species of microalgae have been cultured through the years but the culturing conditions and downstream process can be very different between cases. In the current thesis a downstream process is designed for two types of microalgae, *Dunaliella salina* and *Chlorella vulgaris*. *Dunaliella salina* is chosen due to its high β-carotene production, it is cultured in a photobioreactor under specific conditions to increase growth and β-carotene production. Natural β-carotene products are in the price range $275-$2,750 per kg, making it an interesting choice of product to sell. *Chlorella vulgaris* is often used in health nutrition and is sold for $46 per kg. A downstream process for microalgae consists of one or more of the following steps: harvesting/dewatering, cell disruption, drying and extracting. Methods for each step are reviewed and eventually the most suitable methods and equipment are used in the downstream process design. A cost analysis is done for the downstream process designs. The cost analysis shows that a downstream process of microalgae in Iceland has a great potential.

Útdráttur

Margar tegundir smáþörunga hafa verið ræktaðar í gegnum tíðina en ræktunaraðstæður og framleiðsluferli geta verið mismunandi á milli verkefna. Þessi ritgerð tekur fyrir hönnun á framleiðsluferli fyrir tvær tegundir af smáþörungum, *Dunaliella salina* og *Chlorella vulgaris*. *Dunaliella salina* varð fyrir valinu vegna hæfileika til mikillar β-karótín framleiðslu, ræktunin fer fram í ljóslífrænu hvarfrými (e. photobioreactor) undir sérstökum aðstæðum sem stuðla að auknum vexti og β-karótín framleiðslu. Náttúrulegar β-karótín vörur eru verðlagðar á bilinu $275-$2.750 á kg, þessi háa verðlagning gerir vörurnar áhugaverðar til sölu. *Chlorella vulgaris* er oft seldur á heilsumarkað fyrir $46 á kg. Framleiðsluferli fyrir smáþörunga samanstendur af einu eða fleiri af eftirtöldum þáttum: Einangrun á smáþörung úr vatni/afvötnun, frumusundrun, þurrkun og útdrætti. Fjallað er um vinnsluaðferðir innan þessara þátta og að lokum eru viðeigandi aðferðir og tækjabúnaður valin inn í hönnunina á framleiðsluferli. Kostnaðaráætlun er gerð fyrir þær hannanir sem lagðar eru fram en þær benda til þess að miklir möguleikar geta falist í því að vinna smáþörung á Íslandi.

"It is not because things are difficult that we do not dare, it is because we do not dare that things are difficult."

- Seneca

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Abbreviations

ASE	Accelerated Solvent Extraction
C.vulgaris	Chlorella vulgaris
CFF	Cross Flow Filtration
D.salina	Dunaliella salina
DW	Dry Weight
FDA	Food and Drug Administration
GRAS	Generally Regarded as Safe
H.pluvialis	Haematococcus pluvialis
HPH	High Pressure Homogenizer
LED	Light Emitting Diode
PBR	Photobioreactor
PEF	Pulse Electric Field
PFE	Pressurized Fluid Extraction
RPM	Rounds Per Minute
SCF	Supercritical Fluid Extraction
SPT	Spiral Plate Technology
TSS	Total Solids in Suspension

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1 Introduction

Process engineering is widely used in different industries including biotechnological, chemical, petrochemical and food industries. The goal is to design, control and/or operate processes for various solutions. It is important to use an optimized process to make it as efficient as possible, well optimized process can minimize the cost and time consumption.

Microalgae have great potential for high value products in pharmaceutical, nutraceutical, cosmetics and food additives but biofuel is getting the most attention at this moment. Biofuel made from microalgae is not yet cost efficient but the production of high value products can be efficient.

The purpose of this study is to design a downstream process for two types of microalgae, with and without a rigid cell wall. The former is the halophile green microalgae *Dunaliella Salina (D.salina)* which has an elastic cell membrane and the latter is the single cell microalgae *Chlorella Vulgaris (C.vulgaris)* which has a rigid cell wall. The downstream process methods will be itemized to harvesting, cell disruption, evaporation, drying and extraction. Some of the most used methods will be reviewed as well as some new comers which make a good case.

The designed downstream processes will be a suggestion for a industrial scale plant for the project GeoChem. GeoChem is an ongoing project at the Center for Systems Biology at the University of Iceland. GeoChem has designed photobioreactors (PBR) surrounded with light emitting diodes (LED) to increase growth rate and carotenoid production. Downstream processes dedicated to different commercial products will be designed; *C.vulgaris* powder, lipid from *D.salina* and *D.salina* powder.

1.1 Objectives of the thesis

- Which methods are most suitable for high value product production?
- How is it best to utilize local energy to the downstream process?
- Is it feasible to produce high value products from the GeoChem culture?

2 Background

2.1 Microalgae culture

2.1.1 Photobioreactors

Microalgae is either cultured in open systems or closed. Microalgae cultivation in open ponds is one of the oldest industrial systems for the production of single-cell protein and β-carotene (Mandalam & Palsson, 1998). The disadvantage of open ponds is how dilute the culture is as well as the fact that it is open to all kinds of external material that is not supposed to be in the culture. PBR are a closed system which has been described as a cultivation system for the production of biomass and some specialty chemicals under axenic conditions (Mandalam & Palsson, 1998). PBR cultivation offers control over nearly all biotechnologically important parameters. A closed system like PBRs offers benefits like: reduced contamination risk, controllable hydrodynamics and temperatures and no CO_2 loss (Pulz O. , 2001). PBR have more innovative potential than open pond systems and seem to be more promising for technical developments (Pulz O. , 2001).

The PBR designed by the GeoChem team is tubular and surrounded with LEDs. The LEDs can be optimized to increase growth rate and carotenoid yield in *D.salina*. By combining red LED (75%) with blue LED (25%) growth at higher total photo flux was achieved, by using the additional blue LED instead of red LED increased β-carotene and lutein accumulation in the microalgae (Fu, et al., 2013)

2.1.2 Advantage of location

The plant is designed to be next to a geothermal power plant. The power plant will be a supplier of CO_2 and inexpensive energy for the production of valuable chemicals. The geothermal power plant utilizes geothermal heat to produce electricity and the microalgae production facility will have similar access to geothermal heat.

2.2 Microalgae

It has been estimated that between 200,000 and several million species of microalgae exist and the biodiversity is tremendous (Norton, Melkonian, & Andersen, 1996). GeoChem is culturing *C.vulgaris* and *D.salina* and therefore they are the main focus of this study. The goal is to build an industrial scale plant and perhaps later on in the process other species of microalgae will be taken into consideration for culturing. Therefore an additional

microalgae is described below as well as the main carotenoid it produces. The additional microalgae is *Haematococcus pluvialis (H.pluvialis)* which is known for its astaxanthin production. *H. pluvialis* will not be taken into consideration in any other way in this thesis except the mention in this chapter for promising potential as an astaxanthin producer.

Chlorella, Dunaliella and *Hamatococcus* are classified as food sources falling into the Generally Regarded as Safe (GRAS) by the United States (U.S.) Food and Drug Administration (FDA) (González-mariño, 2010). *Chlorella*, *Dunaliella* and *Haematococcus* all belong to a division of green algae called *Chlorophyta*. *Chlorophyta* is one of two major groups from green algae, the other group is *Conjugaphyta*. *Conjugaphyta* is almost five times larger than the *Chlorophyta* but none of the *Conjugaphyta* has been employed for biotechnological applications (Pulz & Gross, 2004). Typical composition of algal lipids is glycerol, sugars, or bases esterified to fatty acids, with carbon numbers in the range of C_{12} to C_{22} (Chacón-Lee & González-mariño, 2010).

In Table 1 the general composition of some popular commodities are compared to *C.vulgaris* and *D.Salina*.

Table 1. General composition of popular commodities and the C.vulgaris and D.salina in % of dry matter (Becker E. W., 1994).

Commodity	Protein	Carbohydrates	Lipids
Baker's yeast	39	38	1
Egg	26	38	28
Meat muscle	43	1	34
Milk	26	38	28
Rice	8	77	2
Soya	37	30	20
Chlorella vulgaris	51-58	12-17	14-22
Dunaliella salina	57	32	6

Mata et al. (2010) made a comparison of lipid content, lipid productivity and biomass productivities of different microalgae species. The comparison, seen in Table 2, indicates that there is a significant composition difference between the various microalgae species.

Table 2. Comparison of lipid content, lipid productivity and biomass productivities of different microalgae species (Mata, Martins, & Caetano, 2010).

Marine and freshwater microalgae species	Lipid content (% dry weight biomass)	Lipid productivity (mg/L/day)	Volumetric productivity of biomass (g/L/Day)	Areal productivity of biomass ($g/m^2/day$)
Ankistrodesmus sp.	24.0-31.0	-	-	11.5-17.4
Botryococcus braunii	25.0–75.0	-	0.02	3.0
Chaetoceros muelleri	33.6	21.8	0.07	-
Chaetoceros calcitrans	14.6–16.4/39.8	17.6	0.04	-
Chlorella emersonii	25.0–63.0	10.3–50.0	0.036–0.041	0.91–0.97
Chlorella protothecoides	14.6–57.8	1214	2.00–7.70	-
Chlorella sorokiniana	19.0–22.0	44.7	0.23–1.47	-
Chlorella vulgaris	5.0–58.0	11.2–40.0	0.02–0.20	0.57–0.95
Chlorella sp.	10.0–48.0	42.1	0.02–2.5	1.61–16.47/25
Chlorella pyrenoidosa	2.0	-	2.90–3.64	72.5/130
Chlorella	18.0–57.0	18.7	-	3.50–13.90
Chlorococcum sp.	19.3	53.7	0.28	-
Crypthecodinium cohnii	20.0–51.1	-	10	-
Dunaliella salina	6.0–25.0	116.0	0.22–0.34	1.6–3.5/20–38
Dunaliella primolecta	23.1	-	0.09	14
Dunaliella tertiolecta	16.7–71.0	-	0.12	-
Dunaliella sp.	17.5–67.0	33.5	-	-
Ellipsoidion sp.	27.4	47.3	0.17	-
Euglena gracilis	14.0–20.0	-	7.70	-
Haematococcus pluvialis	25.0	-	0.05–0.06	10.2–36.4
Isochrysis galbana	7.0–40.0	-	0.32–1.60	-
Isochrysis sp.	7.1–33	37.8	0.08–0.17	-
Monodus subterraneus	16.0	30.4	0.19	-
Monallanthus salina	20.0–22.0	-	0.08	12
Nannochloris sp.	20.0–56.0	60.9–76.5	0.17–0.51	-
Nannochloropsis oculata.	22.7–29.7	84.0–142.0	0.37–0.48	-
Nannochloropsis sp.	12.0–53.0	37.6–90.0	0.17–1.43	1.9–5.3
Neochloris oleoabundans	29.0–65.0	90.0–134.0	-	-
Nitzschia sp.	16.0–47.0	-	-	8.8–21.6
Oocystis pusilla	10.5	-	-	40.6–45.8
Pavlova salina	30.9	49.4	0.16	-
Pavlova lutheri	35.5	40.2	0.14	-
Phaeodactylum tricornutum	18.0–57.0	44.8	0.003–1.9	2.4–21
Porphyridium cruentum	9.0–18.8/60.7	34.8	0.36–1.50	25
Scenedesmus obliquus	11.0–55.0	-	0.004–0.74	-
Scenedesmus quadricauda	1.9–18.4	35.1	0.19	-
Scenedesmus sp.	19.6–21.1	40.8–53.9	0.03–0.26	2.43–13.52
Skeletonema sp.	13.3–31.8	27.3	0.09	-
Skeletonema costatum	13.5–51.3	17.4	0.08	-
Spirulina platensis	4.0–16.6	-	0.06–4.3	1.5–14.5/24–51
Spirulina maxima	4.0–9.0	-	0.21–0.25	25
Thalassiosira pseudonana	20.6	17.4	0.08	-
Tetraselmis suecica	8.5–23.0	27.0–36.4	0.12–0.32	19
Tetraselmis sp.	12.6–14.7	43.4	0.30	-

2.2.1 Chlorella Vulgaris

Chlorella is a green spherical unicellular microalgae with a rigid cell wall. It contains the green pigment chlorophyll-a and chlorophyll-b. *Chlorella* lives in fresh water and has a cell diameter of 2-10 microns. *Chlorella* was produced by more than 70 companies in 2006 where the largest manufacturer, Taiwan Chlorella Manufacturing and Co, produced over 400 tons of dried biomass per year (Spolaore, Joannis-Cassan, Duran, & Isambert, 2006). The most crucial substance in *Chlorella* is β-1,3-glucan because of its immunostimulant activity, free radical scavenger and the ability to reduce blood lipids (Spolaore et al., 2006). Chlorella has been used as a food additive as a taste and flavor adjusting actions of its coloring agents (Yamaguchi, 1997).

2.2.2 Dunaliella salina

D.salina is a unicellular green microalgae which belongs to the division of *chlorophyta* and the *Dunaliella* genus which lives in saline water. Michel Felix Dunal was the first one to describe *Dunaliella* which he found in Montpellier, France in 1838 but at this time it wasn‘t known as *Dunaliella*. It was in the year 1906 that *Dunaliella* was named in the honor of Dunal. There are many species of *Dunaliella* but the main focus here is *D.salina* because of its known β-carotene production (Oren, 2005). Not all *Dunaliella* species are able to produce large amounts of carotene but those who do, including *D.salina*, do so under suitable condition. These conditions are i.e. high light intensity, limitation of nutrients (Oren, 2005) and high salinity which also increased the β-carotene to chlorophyll ratio (Ben-Amotz & Avron, 1983). The cells size varies because of different shapes of the cell. The length varies between 5-25 µm and the width from 3-13 µm (Tafreshi & Shariati, 2009). *D.salina* can reach β-carotene content up to 14% of dry weight (Metting Jr, 1996). This high β-carotene production in *D.salina* has made biotechnological companies interested in using the microalgae as a source for β-carotene. It was back in 1966 in the USSR when the first pilot scale cultivation of *D.salina* for β-carotene production was established (Oren, 2005). *D.salina* is cultured in various cultivation systems. From low tech open ponds to high tech PBR where the cultivation parameters are controllable to achieve higher productivity and carotene yield. *D.salina* lacks a rigid cell wall but has an elastic cell membrane and is able to grow in wide range of salt concentrations (Karni & Avron, 1988). It has great environmental adaption and produces β-carotene and glycerol to maintain its osmotic balance. The lipid composition of the halotolerant microalgaes, *D.salina* and *Dunaliella bardawil* (*D.bardawil*) were studied by (Vanitha, Narayan, Murthy, & Ravishankar, 2007). Total lipid content was 7.2% dry weight in *D.salina* and 8% dry weight in *D.bardawil*. The composition of the lipids in *D.salina* and *D.bardawil* is shown in Table 3.

Table 3. Lipid composition in D.salina and D.bardawil according to Vanitha et al (2007).

Fraction	D. bardawil (%)	D. salina (%)
Neutral lipids	35.35	29.05
Polar lipids	24.24	42.52
Glycolipids	40.42	28.43

The *D.salina* cell includes oily globules. The globules are nearly only composed of neutral lipids where more than half is β-carotene (Sarmad, Shariati, & Tafreshi, 2006).

2.2.3 Haematococcus Pluvialis

H.pluvialis is a fresh water unicellular green microalgae which belongs to the division of *Chlorophyte* and *Haematococcus* genus. The microalgae is distributed all around the world but mainly in temperate regions (Dore & Cysewski, 2003). *H.pluvialis* is green under optimal growth condition but when stressed with nutrient limitation, high light intensity, high salinity or other unsuitable environmental conditions it forms a spore, non-motile and large resting cell (Boussiba & Vonshak, 1991). When in form of a spore astaxanthin produces rapidly and the color of the spore turns red, the astaxanthin production is thought to be a protective barrier against ultraviolet rays. The spore is able to turn back into a green cell after years of dormancy when conditions are suitable for growth again (Dore & Cysewski, 2003). When *H.pluvialis* is in the form of spore it is feasible to use in astaxanthin production due to the amount of astaxanthin accumulated. The spore has a tough rigid cell wall which has to be disrupted before it is possible to get efficient utilization of the astaxanthin inside and to increase digestibility (Sommer, Potts, & Morrissy, 1991). Production of astaxanthin in *H.pluvialis* can accumulated up to 3% astaxanthin in dry weight (Lorenz & Cysewski, 2000). *H.pluvialis* is a primary source of astaxanthin used in aquaculture and the food industry as well as having the highest accumulation of astaxanthin in nature (Yang, et al., 2011).

2.3 Valuable biochemicals in microalgae

2.3.1 Carotenoids

There are over 700 natural lipid-soluble pigments in the group of carotenoids and most of them are produced within phytoplankton, algae and plants. These pigments can be found in many variations in nature such as colors, most recognizable are the yellow, orange and red, of fruits, leaves and aquatic animals (Lorenz & Cysewski, 2000). Animals are not capable of synthesizing carotenoids like plants, algae and some fungal and therefore need to utilize

carotenoids through a carotenoid rich diets (Davies, 1985). Carotenoids are divided to carotenes and xanthophylls which will be demonstrated further below.

Carotene

β-carotene is a pigment which has antioxidant activities and is a source of pro-vitamin A (retinol). One molecule of β-carotene can be cleaved into two molecules of vitamin A (Biesalski, Chichili, Frank, Lintig, & Nohr, 2007). Liver enzymes oxidize β-carotene to produce vitamin A (Tafreshi & Shariati, 2009). Natural β-carotene from *D.salina* is a vitamin A precursor (Pulz & Gross, 2004). It is possible to overdose on β-carotene with excessive consumption but it is physically harmless with the possibility of the skin turning orange, a state called carotenosis (Stahl, et al., 1998). The human body will use as much as it needs of the vitamin A from the β-carotene, if there is enough of vitamin A the conversion of β-carotene will decrease. High doses of synthetic β-carotene has been linked to higher risk of cancer in smokers i.e. lung cancer and prostate cancer as well as intracerebral hemorrhage and cardiovascular diseases (National institues of health, 2011). β-carotene has been used to reduce the risk of known disorders such as age related macular degeneration (AMD) as well as being thought to reduce the risk of some types of cancer but that has not been proved.

Xanthophill

Natural **astaxanthin** can be derived from the microalgae *H.pluvialis* but before that is was derived from crayfish, krill and the red yeast *Xanthophyllomyces dendrorhous* also known as *Phaffia rhodozyma* (Dore & Cysewski, 2003).

The largest market for astaxanthin is in aquaculture where it is used as an addition to the fish feed. It colors the muscle of the salmon and trout red, making it more desirable to the customer. More than 90% of astaxanthin sold is synthetic, all-trans (Spolaore et al., 2006).

Astaxanthin has great antioxidant effects and in some cases it has much stronger free radical antioxidant activity than vitamin E and β-carotene. Some biological functions of astaxanthin include antioxidant for polyunsaturated fatty acids, protection of UV light, pigmentation as well as improved reproduction (Guerin, Huntley, & Olaizola, 2003).

2.4 Commercial products

Microalgae biomass has been used in the health food industry for the past decades. The most popular production of the biomass is to manufacture powder, tablets and capsules, this counts for more than 75% of the annual biomass production (Pulz & Gross, 2004)**.** Microalgae can be incorporated into products like pasta, noodles, bread, gum, snack foods and drinks (Yamaguchi, 1997).

Spirulina and Chlorella have been dominating the algae market as can be seen in Table 4, annual production of 3,000 t dry weight and 2,000 t dry weight respectively.

Table 4. Annual production and prices of microalgae products presented by (Brennan & Owende, 2010).

Microalgae	Annual production	Producer country	Application and product	Price ($)
Spirulina	3,000 t dry weight	China, India, USA, Myanmar, Japan	Human nutrition	46 kg^{-1}
			Animal nutrition	-
			Cosmetics	-
			Phycobiliproteins	14 mg^{-1}
Chlorella	2,000 t dry weight	Taiwan, Germany, Japan	Human nutrition	46kg^{-1}
			Cosmetics	-
			Aquaculture	64 l^{-1}
Dunaliella salina	1,200 t dry weight	Australia, Israel, USA, Japan	Human nutrition	-
			Cosmetics	-
			β-carotene	275-2,750 kg^{-1}
Aphanizomenon flos-aquae	500 t dry weight	USA	Human nutrition	-
Haematococcus pluvialis	300 t dry weight	USA, India, Israel	Aquaculture	50 l^{-1}
			Astaxanthin	7,150 kg^{-1}
Crypthecodinium cohnii	240 t DHA oil	USA	DHA oil	55 g^{-1}
Shizochytrium	10 t DHA oil	USA	DHA oil	55 g^{-1}

The main products and application areas for *C.vulgaris*, *D.salina* and *H.pluvialis* are shown in Table 5.

Table 5. The main products from C.vulgaris, D.salina and H.pluvialis and suitable application areas (Pulz & Gross, 2004).

Microalgae	Product	Application areas
Chlorella vulgaris	Biomass	Health food, food suppliment, feed surrogates
Dunaliella salina	Carotenoids, β-carotene	Health food, food suppliment, feed
Haematococcus pluvialis	Carotenoids, Astaxanthin	Health food, pharmaceuticals, feed additives

2.4.1 Chlorella

Biomass is dried and marketed in the form of powder and tablets for health nutrition. It is used as a protein supplement but is thought to have other health benefits such as cancer prevention, immune system support and weight control (Belasco, 1997). *C.vulgaris* extract is used to support tissue regeneration and reduce wrinkles by stimulating collagen synthesis in skin (Spolaore et al., 2006).

2.4.2 Dunaliella salina

Cognis Nutrition and Health is the largest producer of *D.salina* powder. The powder is used as an ingredient of dietary supplements and functional foods (Spolaore et al., 2006). Synthetic β-carotene is far less expensive than natural β-carotene, that is because natural β-carotene from *D.salina* supplies natural isomers in their natural ratio (García-González, Moreno, Manzano, Florencio, & Guerrero, 2005). It is acknowledged today that natural isomer of β-carotene is superior to the synthetic all-trans form (Radmer, 1996). There are three different main products derived from *D.salina* and they are β-carotene extracts, *D.salina* powder for human consumption and *D.salina* powder for feed. The price for natural β-carotene varies according to specifications and demand, from \$300-\$3,000 kg^{-1}, pricing from 2004 (Ben-Amotz, 2004) which is similar to the \$275-\$2,750 stated above.

β-carotene extract

The purified β-carotene extract is usually mixed with vegetable oil in bulk concentrations from 1% to 20%. This is used to color various food products or put into soft capsules for human consumption. Usually each capsule contains 5 mg β-carotene and often a carotenoid mix from *D.salina* is included as well. The carotenoid mix includes predominantly lutein, neoxanthin, zeaxanthin, violaxanthin, cryptoxanthin, α-carotene comprising approximately 15% of the carotene concentration. Variations of this formula are found under market sections of vitamins, health food or food supplement (Ben-Amotz, 2004).

D.salina powder for human consumption

D.salina powder is sold in the form of tablets which are coated with sugar or hard capsules which are packed separately in aluminum/polyethylene blisters prevent oxidation and extend the shelf life of the product. There is between 3 mg and 20 mg mix of 9-cis and all-trans β-carotene per unit of these products. This powder is low salt and the process must include a washing of salt (Ben-Amotz, 2004).

D.salina powder intended for feed

D.salina powder sold as feed colorant and pro-vitamin A use in cattle, poultry, fishes, shrimps and more. There is about 2% β-carotene in the powder but the salt concentration

varies, the process has no washing of salt. The powder is shipped under vacuum in aluminum/polyethylene bags (Ben-Amotz, 2004).

Following is a range of β-carotene products (Borowitzka & Borowitzka, 1990):

- 4% β-carotene solution used in dietary supplement industries as a fill for soft gelatin capsules.
- 1.5-30% oil (e.g. soybean oil) solution used as food colorant (mainly for margarine) and dietary supplement.
- 2% emulsion used in beverages.
- β-carotene rich *D.salina* powder containing 2-5% β-carotene utilized as aquiculture feed for prawns and certain other species or packed in capsules or tablets and labeled as „Natural β-carotene“.
- Water dispersible β-carotene powder and mixed carotenoids.

2.4.3 Haematococcus pluvialis

Astaxanthin is used in aquaculture industry primarily as salmon feed. Synthesized astaxanthin, all-trans, is dominating nearly all markets over natural astaxanthin, 3S and 3‘S, due to the high price of natural astaxanthin (Guerin, Huntley, & Olaizola, 2003). Carp, chicken and red sea bream diets prefer natural astaxanthin due to consumer demands for natural products, regulatory requirements and the fact that the tissue will absorb the natural astaxanthin (Cysewski & Lorenz, 2004). Astaxanthin from H. pluvialis is used in aquaculture to color muscles in fish, especially to color the salmon muscle beautifully red (Pulz & Gross, 2004).

3 Downstream process

3.1.1 Harvesting

The most common harvesting methods are centrifugation, filtration, flotation and sedimentation where flocculation is often recommended as a pretreatment (Mata, Martins, & Caetano, 2010). Most microalgae cells are small and have the size range of 5-50 µm. Microalgae cells have a negative charged surface that hinder aggregation making it harder to harvest. Large volume of suspension has to be handled to recover biomass because of how dilute the suspension is.

There is no universal harvesting method suitable for every case and species. Harvesting requires one or more solid-liquid separation methods and can contribute 20%-40% of the total cost of biomass production (Molina Grima, Belarbi, Acién Fernández, Robles Medina, & Chisti, 2003). The preferable harvesting method is chosen according to attributes of the microalgae and the final product. There are thousands of microalgae species and they vary in many ways and there are unique challenges for each algal species due to difference in shape, size, density, cell surface, charge and culturing conditions such as salinity and pH level. To achieve an efficient algal separation process it should be able to process large volume of broth, yield a product with high dry weight percentage, require modest investment, low energy consumption and low maintenance cost (Poelman, Pauw, & Jeurissen, 1997). When producing high value products it is possible to use more expensive equipment to ensure purity and better efficiency in less time.

Gravity Sedimentation

Gravity sedimentation is a solid-liquid separation where particles are separated from liquids due to settling characteristics. These characteristics are determined by density difference between particles and fluid, particles radius and sedimentation velocity. Gravity sedimentation is the most commonly used harvesting method in waste water treatment of low value algae biomass due to the large volume treated (Molina Grima et al., 2003). Natural gravity sedimentation (without any added chemicals) is not suitable for small microalgae (<70 µm) (Brennan & Owende, 2010) because of settling velocity. The net force acting on the particle controls the sedimentation rate. Stokes Law is used to describe sedimentation rate for inert spherical isolated particles in a Newtonian fluid.

$$V = \frac{g(\rho_{s-}\rho_f)d^2}{18\mu}$$

Where V is the terminal velocity (m s^{-1}), g is the acceleration due to gravity (m s^{-2}), ρ_s is the density of the particle (kg m^{-3}), ρ_f is the density of the fluid (kg m^{-3}), d is the diameter of the spherical particle (m) and μ is the fluid viscosity (kg m^{-1} s^{-1}) (Pahl, et al., 2013).

It is often suitable to add flocculants to the suspension to increase settling velocity with aggregated particles (Molina Grima et al., 2003), when the particles have aggregated it is referred to them as „Flocs". Flocs contain water and have a complicated structure. The density, radius and shape of the floc are undefinable therefore the sedimentation rate can no longer be described with Stokes Law (Shelef, Sukenik, & Green, Microalgae Harvesting and Processing: A Literature Review, 1984). It is possible to use Lamella separators and sedimentation tanks for gravity sedimentation. Sedimentation tanks are an inexpensive process but without addition of flocculants the reliability is low (Shelef et al., 1984). Lamella separators offer increased settling rate due to the incline of the Lamella plates (Mohn, 1980). The particles settle on the inclined plates and flow downwards while water flows upwards. It is possible to add flocculants to increase settling further in the Lamella separator. Gravity sedimentation is usually used for low-value products and can be found in sewage processes. The advantage of gravity sedimentation is the low cost and energy requirements but the disadvantages is the slow sedimentation rate and low solids concentration achieved (Pahl, et al., 2013).

Centrifugation

Centrifugation is a solid-liquid separation based on sedimentation but enhances the settling rate by centrifugal force. The centrifugal force will speed up the separation process. The recovery depends on the settling characteristics of the particles, time in the centrifuge and settling depth. Centrifugal separation is based on density difference of the particle and the surrounding medium. The particle will move outwards when denser than the medium but inwards when less dense. Behavior of the smallest particles affects the separation efficiency the most (Shelef et al., 1984). The relative centrifugal force (RCF) describes the acceleration applied to the centrifuged material. The acceleration is often measured in multiples times the gravitational force of earth (g). The equation for the relative centrifugal force is presented below.

$$RCF = 1.118 * 10^{-5} * r * N^2$$

Where RCF is the relative centrifugal force (g), r is the rotor radius (cm) and N is the rotation speed (RPM).

Centrifugation is a rapid process but energy intensive and therefore it might not be suitable for low value products. Most microalgae can be harvested with centrifugation but the centrifugal force and time can vary between species due to how shear sensitive they are. Centrifugation is a good choice of recovery for production of extended shelf-life

concentrates for aquaculture hatcheries and nurseries (Heasman, Diemar, O'Connor, Sushames, & Foulkes, 2000).

Mohn (1980) performed a study that compared various types of machinery with centrifugal force, mostly centrifuges, such as a self-cleaning disc-stack centrifuge, a nozzle discharge centrifuge, a decanter bowl centrifuge and a hydrocyclone which is not a centrifuge but uses centrifugal force. The comparison is shown in Table 6.

Table 6. Comparison of centrifugal methods of harvesting of microalgae based on Mohn (1980).

Machine and maker	Operational mode	Concentration method	Algae	TSS (%)	Energy (kWh) consumed per m^3	Reliability
Self-cleaning disc-stack centrifuge; Westfalia	Suspension continuous; concentrate discontinuous	One step	Scenedesmus, Coelastrum proboscideum	12	1	Very good
Nozzle discharge centrifuge; Westfalia	Continuous	For final concentration and pre-concentration	Scenedesmusm, C. Proboscideum	2-15	0.9	Good
Decanter bowl centrifuge; Westfalia	Continuous	For final concentration	Scenedesmusm, C. proboscideum	22	8	Very good
Hydrocyclones; AKW	Continuous	For pre-concentration	C. proboscideum	0.4	0.3	Poor

Alfa Laval gives guidance in Figure 1 for choosing suitable harvesting equipment according to TSS and particle size.

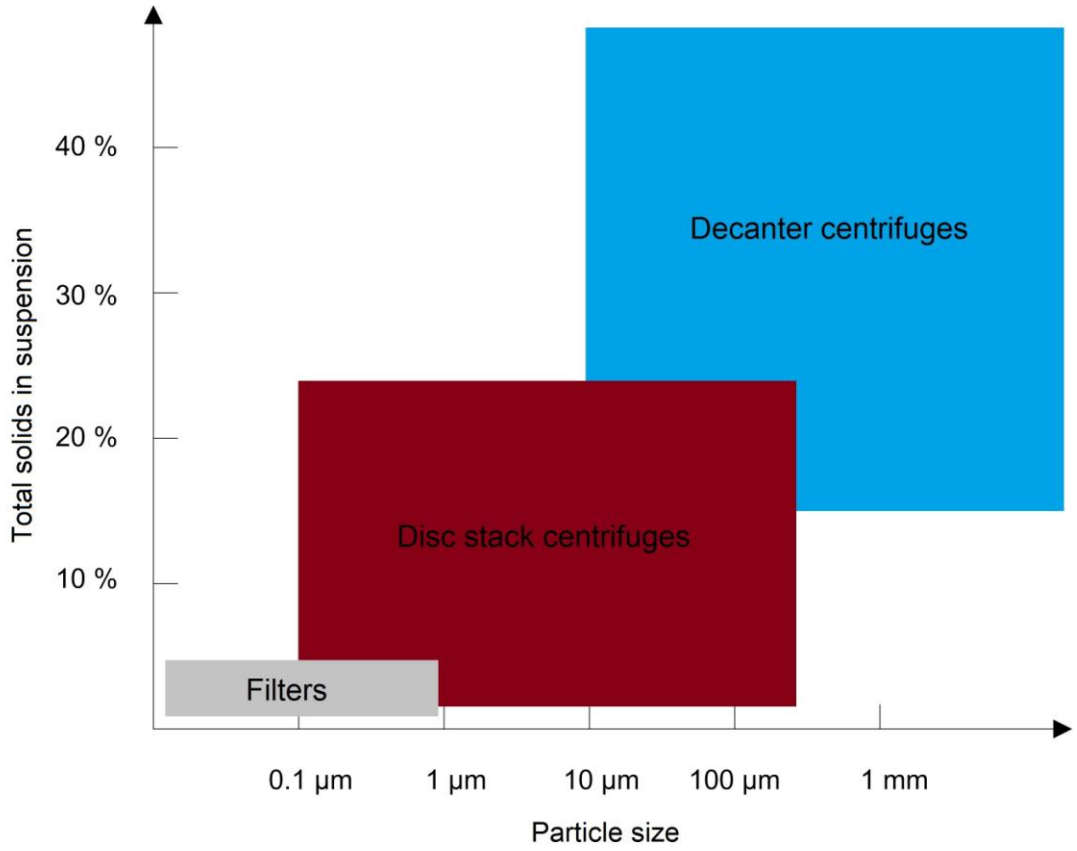

Figure 1. A graphic view demonstrating which equipment is suitable for particular particle size and the TSS (Alfa Laval, 2013).

Advantages of using centrifugation are the possibility of processing large volume of broth in a short time, maintaining the viability of the cell as well as containing the biomass and therefore reduce risk of contamination (Molina Grima et al., 2003).

A clarifier and purifier are types of disc stack centrifuges with one main difference, clarifier is a solid-liquid separation but purifier is a solid-liquid-liquid separator. The difference is shown in Figure 2 and Figure 3.

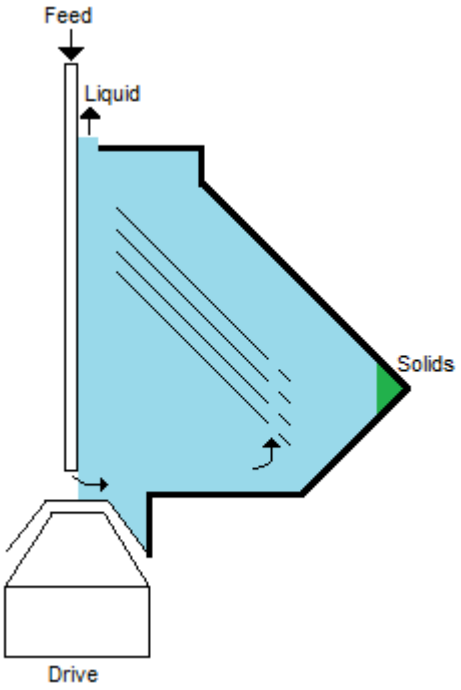

Figure 2. A clarifier gathers solids in the far end and the liquid flows upwards.

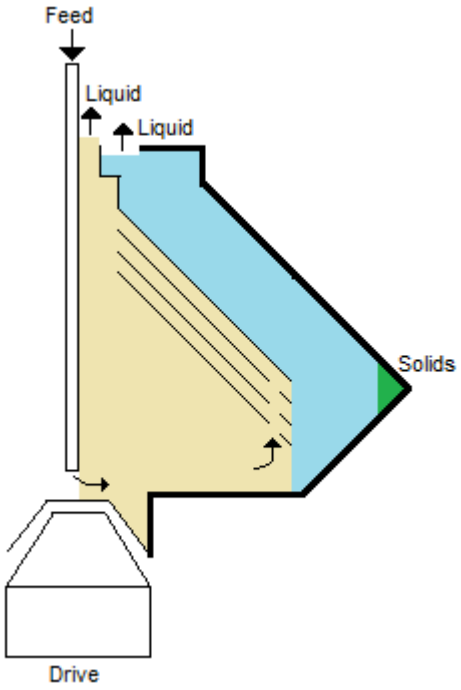

Figure 3. A purifier gathers solids in the far end and has two liquid streams upwards.

Spiral plate technology

The company Evodos has designed a new type of centrifugation harvesting method, the spiral plate technology (SPT). The Evodos equipment uses a drum, shaft and SPT plates in harvesting, all of these parts run at the same velocity preventing any friction between the components. The SPT plates pivot outwards until they rest with their outer long side against the drum. The cylindrical drum runs coaxially against the SPT plates but at the same speed. This method is good for shear sensitive microalgae. The artificially gravity applied to the suspension is 1700-3000 *g*. Heavier components in the suspension will move away from the center of the Evodos settler. Due to the spiral shape of the plates it only has to travel a short distance to meet the SPT plates where the material either sticks to the SPT plate or it moves as far as possible along the plate (Evodos, 2013).

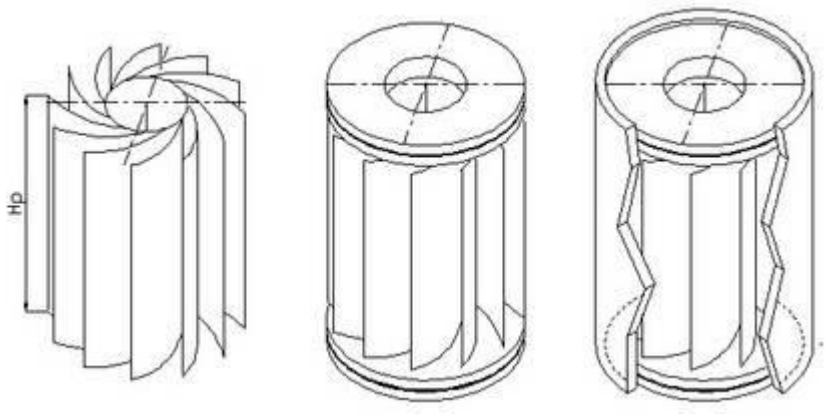

Figure 4. Spiral plate technology designed by Evodos.

Flocculation

Flocculation is the process where solute particles aggregate after collision with each other and form so called Flocs (Uduman, Qi, Danquah, Forde, & Hoadley, 2010). Flocculation is often used as a pretreatment for other harvesting methods. Usually chemicals are added to induce flocculation, those chemicals are called flocculants. Flocculants should be chosen so it will have the least affect on the upcoming downstream process. It is possible to process high volumes of suspension with flocculants in a relatively cheap matter but the flocculation efficiency is highly dependent on species and growth stage. No clear correlation has been proved between flocculants dosage, flocculation efficiency and algal taxonomic group (Williams & Laurens, 2010). It depends on the final product whether flocculants are suitable. If the biomass is used for food or feed the use of certain flocculants such as $AlOH_3$ and some polymers may not be suitable due to risk of toxicity. If the biomass is extracted the final product from the extract might not be contaminated but the co-products, defatted biomass, could still be contaminated (Borowitzka M. A., 1992). The two main types of flocculants are inorganic flocculants and polyelectrolyte flocculants also known as organic polymer. Autoflocculation is another type of flocculation without addition of chemicals. Optimal dosage and pH levels for inorganic and polymeric flocculants are shown in Table 7 (Shelef et al., 1984).

Table 7. Different flocculants and their optimal dose and pH for microalgae flocculation (Shelef, Sukenik, & Green, 1984).

Type or Class	Flocculant	Optimal Dose (mg/L)	Optimal pH
Inorganic	Alum	80-250	5.3-5.6
	Ferric sulfate	50-90	3.0-9.0
	Lime	500-700	10.5-11.5
Polymeric	Purifloc	35	3.5
	Zetag 51	10	>9
	Dow 21M	10	4.0-7.0
	Dow C-31	1-5	2.0-4.0
	Chitosan	100	8.4

Polyelectric flocculants are both natural and synthetic polymers that include ionic and non ionic species. Polymer flocculants can be anionic, cationic or nonionic. Flocculation by polymer flocculation happens because of charge neutralization and particle bridging. Polymer molecules attach on the particles surface by electrostatic and chemical force. The attachment is due to one of or a combination of Coulomb (charge-charge) interactions, dipole-dipole interactions, hydrogen bonding or van der Waals interactions (Uduman et al., 2010). The polymers can stick to the microalgae particle and form a tail. The tails can attach to similar parts of polymers and form bridges between the particles and aggregate the microalgae (Tenney, Echelberger, Schuessler, & Pavoni, 1969). Inadequate bridging happens when the particle is covered with less than the optimum amount of polymers and the few polymers can‘t withstand the shear force. On the other hand if there is too much coverage it can hinder bridging (Tenney et al., 1969).

By increasing the molecular weight of the polymeric flocculants the optimal dose required can be lowered (Tilton, Murphy, & Dixon, 1972). Microalgae surface is negatively charged and neutralization is the basis for microalgae flocculation. Therefore anionic and nonionic polyelectrolytes are not good flocculants for microalgae if solely used (Uduman et al., 2010). The most effective flocculation with cationic polyelectrolytes was achieved at low pH levels (Tenney et al., 1969). To achieve effective sedimentation, Flocs need to excess 100 µm in size (Uduman et al., 2010).

The marine environment has a high ionic strength and the effect of ionic strength on the flocculation with cationic polymers has been studied in general (Tricot, 1984) as well as for microalgae (Sukenik, Bilanovic, & Shelef, 1988). The high salinity prevents flocculation with polymers, effective flocculation was achieved with salinity levels lower

than 5 g/L. Polymers will fail to bridge between particles in high ionic strength due to reduction in size to its smallest dimension (Bilanovic, Shelef, & Sukenik, 1988).

Inorganic flocculants use charge neutralization to flocculate. Charge neutralization is accomplished when microalgae particle has adsorbed an equal amount of the opposite charge leading to cancellation of the net electrical charge. The microalgae must be small and spherical to induce flocculation with inorganic flocculants. Inorganic flocculants can flocculate microalgae when pH level is low enough to form hydrolysis products (Uduman, Qi, Danquah, Forde, & Hoadley, 2010). Alum, ferric sulfate and lime are inorganic flocculants used for microalgae flocculation (Shelef et al., 1984). Large amount of lime is required to induce flocculation and the sludge formed contained a greater amount of lime than microalgae. Alum has better flocculation ability than ferric sulfate in terms of optimal dose of flocculant, pH level and quality of the water slurry obtained (Uduman et al., 2010).

Using alum and ferric chloride has been studied for flocculating marine microalgae. The optimal dose required to flocculate marine microalgae was found to be about five to ten times higher than for freshwater microalgae. The high salinity reduces the chemical activity of the flocculant and masks the functionally active sites which prevents the flocculation. The optimal dose for these two flocculants increased significantly when the ionic strength increased in the media (Sukenik, Bilanovic, & Shelef, 1988).

Combined flocculation process consists of more than one flocculant. The first approach was to use inorganic and polymer flocculants and preceding the flocculation process with oxidants. The addition of chitosan (polymeric) and alum or ferrit chloride (inorganic) improved the flocculation and reduced the required dosage of inorganic flocculant. Chitosan failed to bridge between particles at high ionic strengths (Sukenik et al., 1988).

Autoflocculation is a self generated flocculation. This happens when pH levels increase due to photosynthetic carbon dioxide consumption of the microalgae and sedimentation of inorganic sediments. The microalgae flocculate because of excretion of organic macromolecules, inhibited release of microalgae daughter cells and aggregation between molecules and bacteria (Uduman et al., 2010).

Figure 5 shows how microalgae particles aggregate in the flocculation process.

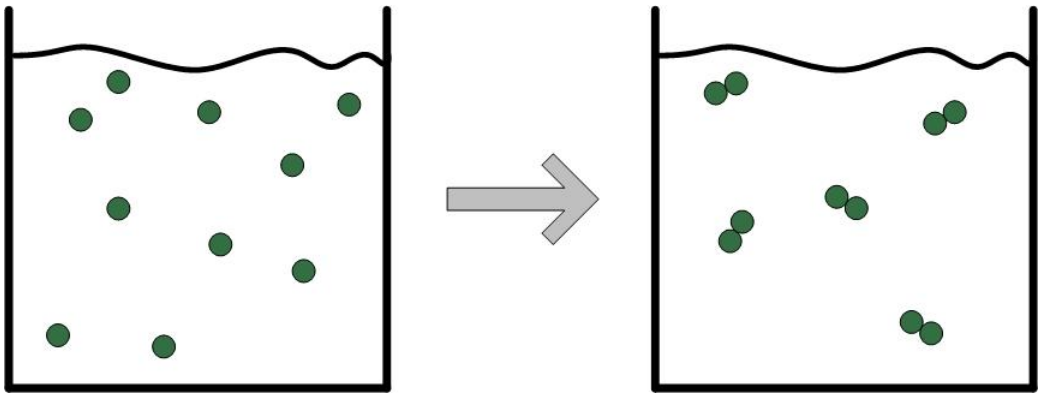

Figure 5. Demonstration of the flocculation process, particles have aggregated on the second picture.

Filtration

There are various filtrations used in harvesting i.e. dead end filtration, microfiltration, ultrafiltration, pressure filtration, vacuum filtration and cross flow filtration. The basics of filtration are to accumulate the desired material, microalgae in this case, and allow the medium to pass through. The suspension will run through the filters until enough algae has accumulated and formed an algae paste (Harun, Singh, Forde, & Danquah, 2010).

It depends on the cell dimension what kind of filtration is adequate, microalgae with a small cell dimension such as *D.salina* and *C.vulgaris* cannot be recovered by pressure or vacuum filtration methods (Harun et al., 2010). Filtration efficiency is highly dependent on the microalgae size and microalgaes with a small cell rapidly clog the filters (Mohn, 1980).

Mohn (1980) compared different types and brands of pressure and vacuum filtrations for dewatering microalgae and found out that pressure belt filter and vacuum filter thickener were not suitable for harvesting. It is not practical to filter small cell of *D.salina* through sand filters, cellulose fibers and other filter materials. Cross flow filtration can be suitable for small microalgae like *D.salina* and *C.vulgaris*.

Cross flow filtration (CFF), also known as tangential flow filtration, is a filtration method where the medium flows tangentially across a membrane. The retentate is looped through the membrane again. It is possible to use ultrafiltration or microfiltration membrane adjustable in pore size or molecular weight cutoffs (Petrusevski, Bolier, Breemen, & Alaerts, 1995). Particles bigger than the membrane pore size will accumulate but particles smaller in size pass through. CFF is considered to be energy efficient considering the output and initial concentration (Danquah, Ang, Uduman, Moheimani, & Forde, 2009). It is possible to harvest shear sensitive suspension with CFF. Using CFF for large scale harvesting might not be adequate because of continuous clogging and replacement of membranes. The major cost for this method is because of membrane replacement and

pumping (Rossignol, Vandanjon, Jaouen, & Quéméneur, 1999). Microfiltration has the pore size 0.1-10 µm and is often used in solid-liquid separation of micron sized particles. Ultrafiltration has the pore size 0.001-0.100 µm, which corresponds to 1000-500000 Da, and is used for separations at molecular level, i.e. pigments, amino acids, enzymes, vitamins, proteins and antibiotics (Rossignol et al, 1999). The difference between dead end filtration and cross flow filtration is demonstrated in Figure 6.

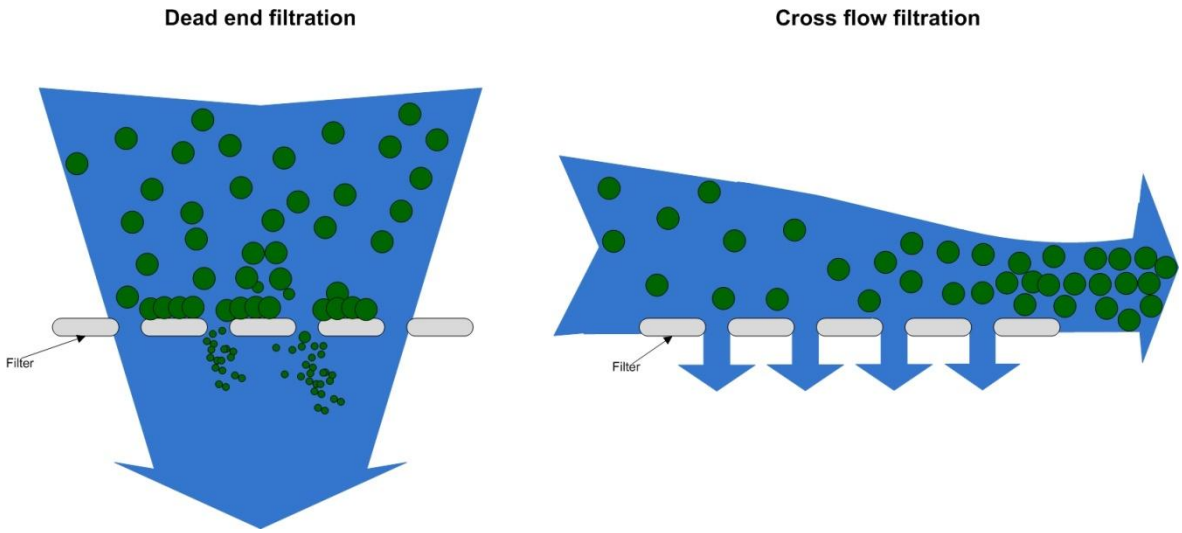

Figure 6. Dead end filtration versus cross flow filtration.

Flotation

Flotation is a separation process depending on air or gas bubbles to attach with solid particles and accumulate as a float. The air, solid and aqueous phases contact angle is also a deciding factor of the attachment of a bubble and an particle (Uduman et al., 2010). The gaseous molecules float through the solid-liquid suspension and attach to the solid particles on the way up. When the solid particles have accumulated with the bubbles, they carry on to the surface where the float is harvested. The lower instability of the suspended particles the higher air-particles contact (Shelef et al., 1984). It is possible to use flotation for particles with diameter of less than 500 µm (Matis, Gallios, & Kydros, 1993), the smaller the particle the larger possibility that the particles can be levitated by the bubbles. With decreasing particle size the probability of collision with a bubble decreases as well (Matis et al., 1993).

Dissolved air flotation is based on fine air bubbles to carry the solid particles to the surface. To create these air bubbles a water stream filled with dissolved air is injected through a nozzle or needle valves at atmospheric pressure into a flotation tank, part of the clarified liquid is recycled back into the flotation with the addition of saturated air (Matis et al., 1993). There are two factors that affect the dissolved air process particularly, concentration of air and the diameter of the bubbles. Diameter of the bubbles vary in size

and can range from 10-100 µm with a mean of 40 µm. Small bubble size can be achieved by using saturator process above atmospheric pressure. Increased pressure on the injection on flow will lead to smaller bubble size (Uduman et al., 2010). The concentration of air can be controlled by changing the saturator pressure or the recycle ratio. A demonstration of the flotation process is shown in Figure 7.

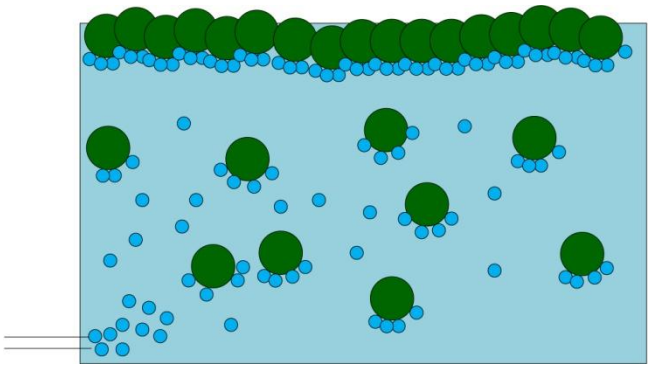

Figure 7. Demonstration of the flotation process where micro bubbles are floating the microalgae particles.

Microstraining

Screening is a harvesting method which passes the suspension through a screen, the screens can have different pore sizes. The screen harvests particles that are larger than the screens pore size, liquid phase passes through. Microstraining is a type of screening. Microstrainers are a rotary drum covered by a screener, a straining fabric, stainless steel or polyester. Particles are collected via backwash onto an axial through. Microstrainers are cleaned periodically (Shelef et al., 1984). The efficiency of a microstrainer depends on the microalgae used as well as the concentration of the suspension, high concentration can block the screen while low concentration can result in inefficient capture. The flow-through rate of the screener is determined by the pore sizes of the screener indicate how much suspension is processed per microstrainer unit (Shelef et al., 1984). Microstrainers use a simple function, consist of simple construction and are easy to operate. The investment is low considering a harvesting method as well as the energy consumption. Disadvantages that can encounter with microstrainers are incomplete solids removal and difficulty handling solids fluctuations, these problems may be partially fixed by varying the rotational speed (Shelef et al., 1984).

Newly developed systems

The **AlgaeVenture Systems** (AVS) method is a solid-liquid separation. They claim to dewater algae slurries with dramatically reduced energy consumption by utilizing surface physics and capillary action. They claim to separate solids with up to 90% less energy than

a centrifuge. Nutrient recovery from the harvesting waste can create valuable co-products. It is possible to dry the algae to flakes directly in the AVS harvesting system.

The AVS method was granted a $6M merit award by the U.S. Department of Energy‘s Advance Research Projects Agency in 2009 (Algaeventure Systems, 2013). Algaeventure Systems claim that the energy cost can be reduce significantly with their system compared to centrifugation, see Table 8.

Table 8. Comparison done by the Algaeventure Systems with 10 micron C.vulgaris (Algaeventure Systems, 2013).

Process	Energy cost	Water remaining after dewatering
Centrifuge	$3.400 / ton	12.500 L
AVS SLS system	$1.92 / ton	5.555 L

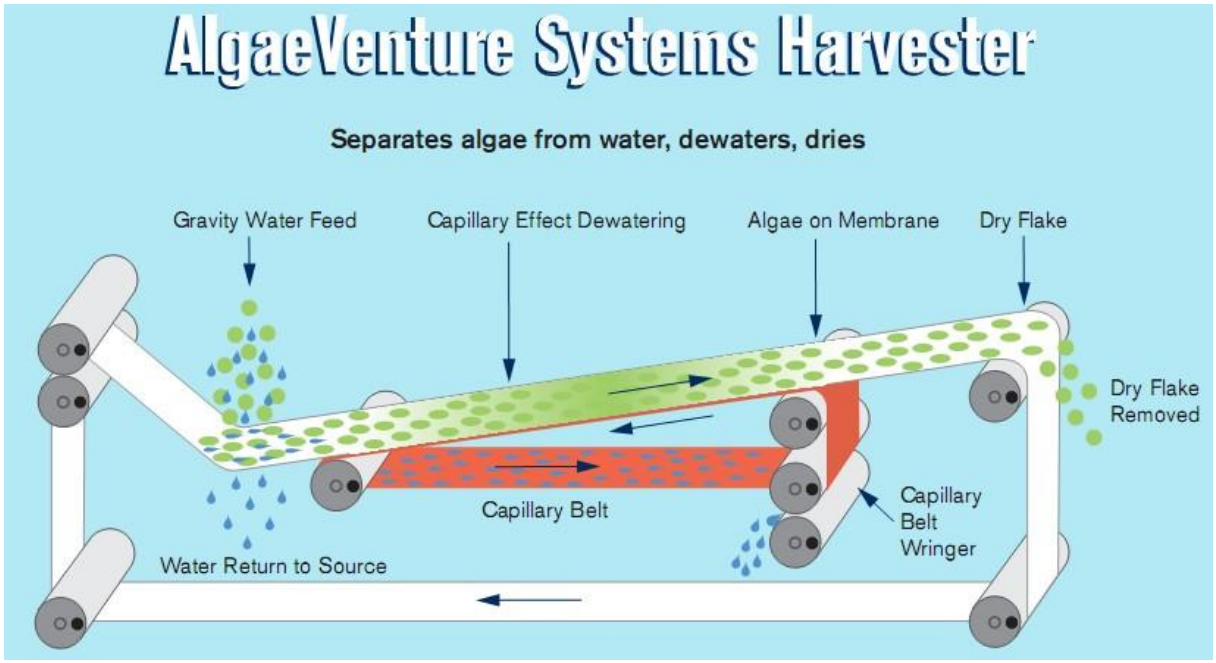

Figure 8. Algaeventure Systems harvesting method (Algaeventure Systems, 2009).

OriginOil has invented a harvesting equipment capable of dewatering and disrupting cell walls all in the same process, called Algae Appliance. This is done chemical free and with low energy consumption. It is possible to pump the suspension straight from the PBR to the Algae Appliance but it might be feasible to pump to a buffer tank. Electromagnetic pulse is used to flocculate and disrupt the cellular walls.

The Algae Appliance model 200 is a pilot scale harvesting system. The flow rate is variable up to 200 L/min, 12,000 L/hour, with the potential to remove up to 95% of the

initial water volume. It can run continuously or intermittently on a wide variety of microalgae strains. It can process 96,000 L/day in continuous harvesting (OriginOil, 2013).

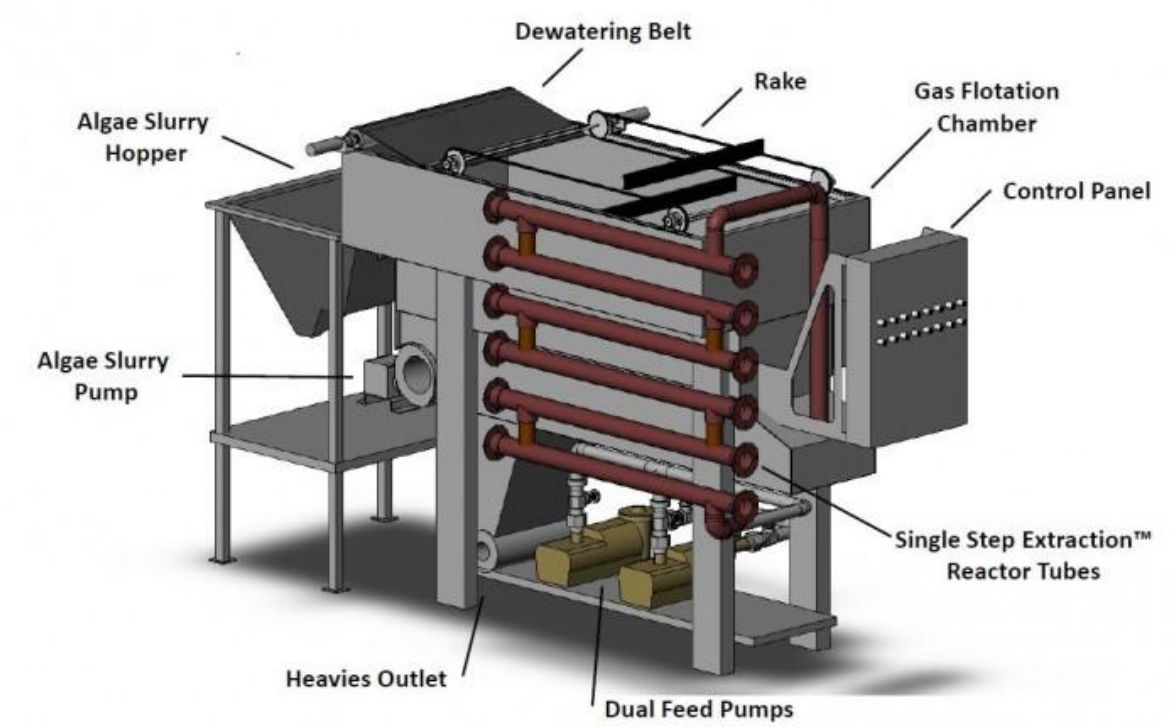

Figure 9. The Algae Appliance model 200 (OriginOil, 2013).

3.1.2 Cell disruption

Microalgae vary in composition, structure and size. One main difference in particularly is the cellular structure. Microalgae such as *D.salina* have an elastic cellular membrane which is easily digestible by humans but others such as *C.vulgaris* have a rigid cellular wall which needs disruption to get to the intraocular products such as oil. Cellular walls can resist high pressure and can be weight for weight as strong as reinforce concrete. It is possible to use many of the cell disruption methods made for non-photosynthetic for microalgae (Chisti & Moo-Young, 1986).

The cell disruption is done to (Molina Grima, González, & Giménez, 2013):

1. Reduce extraction time.
2. Reduce solvent use.
3. Avoid use of high temperature and pressure forcing the solvent in touch with the lipids.
4. Make easy contact with intracellular lipids and solvents which leads to increased lipid yield.

Bead milling

Bead mill uses kinetic energy to force small beads to collide with each other. The kinetic energy is formed with discs which are fitted to a rotating shaft in the center of a grinding cylindrical chamber. The beads create a high shear force by forming stream layers of different velocity. The suspension is pumped into the grinding chamber by an external pump. Cell disruption by bead milling is possible because of collision between beads and cells. There are number of beads in the suspension and by rapidly stirring in the suspension the beads will crush the cell membrane or cell wall (Williams & Laurens, 2010). Bead milling is noted as one of the most efficient techniques for physical cell disruption. The chambers can be horizontal or vertical, the horizontal configuration is known to be more disruption efficient (Chisti & Moo-Young, 1986). The amount of disruption depends mostly on the collision between beads and cells, strength of the microalgae cell wall and size, shape and composition of the beads (Doucha & Lívanský, 2008). This type of cell disruption is generally used in conjunction with solvents to recover (Mercer & Armenta, 2011).

High pressure homogenization

High pressure homogenizer (HPH) pumps the suspension with high pressure of 150 MPa up to 400 MPa through an orifice which results in immediate pressure change and high shear force on to a valve seat. The collision with the valve seat leads to a further collisions of cells with an impact ring. HPH is used in dairy industry (Geciova, Bury, & Jelen, 2002) and for the disruption of microorganisms (Anand, Balasundaram, Pandit, & Harrison, 2007). Reasons for cell disruption with HPH differ between theories; because of collision with hard surface, turbulence, viscous and high pressure shear, pressure drop, decreased flow velocity and cavitations (Lee, Lewis, & Ashman, 2012). For heat sensitive products it is recommended to add a cooling process since temperature is expected to rise about 2 °C per pass for every 10 MPa applied in the HPH (Becker, Ogez, & Builder, 1983).

Sonication

Sonication is a liquid-shear method using frequencies around 25 kHz, ultrasound, to create cavitations in the suspension. Cavitations is when micro bubbles form because of high acoustic power input, grow during the rarefaction phase of the sound wave and releasing a shock wave when they collapse after a compression which disrupts surrounding materials in the suspension. When the bubbles collapse sonic energy is transformed to mechanical energy in the form of elastic waves (Chisti & Moo-Young, 1986). Sonication can disrupt cells at relatively low temperature when compared to other cell disruption methods i.e. autoclave and microwave. By using lower temperature it will lead to less thermal protein denaturation. Sonication can disrupt cells without the addition of chemicals or beads (Gerde, Lomboy, Yao, Grewell, & Wang, 2012) but to disrupt tougher material it is

possible to add glass beads or quartz sand to enhance the disruption efficiency (Wiltshire, Boersma, Möller, & Buhtz, 2000). To induce sonication two types of sonicators are used, horns and baths (Hosikian, Lim, Halim, & Danquah, 2010). These two types are used for batch operations but can be used for continuous operations with the addition of flow cells. Sonication energy scatters away from the source limiting the maximum effective volume of disruption (Lee, Lewis, & Ashman, 2012).

Horns made out of titanium are used to amplify a vibration at 10 µm - 15 µm to 100 µm - 150 µm amplitude at the tip of the horn. The vibration source is a piezoelectric generator made of lead zirconate titanate crystals. To successfully form cavitations with disruptive force the tip of the horn must have high power intensity. If horn sonication is to be used on industrial scale it is necessary to form a clustering of multiple horn units to form disruptive forces to the whole volume of medium (Lee et al., 2012).

Sonicator **baths** generate ultrasonic waves inside a reactor using transducers. The transducers vary in capacity and it is possible to use number of transducers at once, depending on the size and shape of the reactor. It is possible to increase disruption efficiency by using multiple transducers with 2 or 3 different frequencies (Lee et al., 2012).

Microwave

Microwaves are high frequency waves of frequencies about 2.5 GHz which generate heat by frictional force from intra- and intermolecular movements (Amarni & Hocine, 2010). Microwaves disrupt the cell from within because of production of water vapors from the heating.

3.1.3 Drying

Drying is a process method to increase the storage time of the biomass. The chosen drying method should be able to remove nearly all moisture without harming the nutritional and biochemical qualities of the alga as well as being economical (Chidambara Murthy, 2005).

Freeze drying, spray drying and drum drying of *D.salina* have produced satisfactory results in terms of stability of β-carotene in biomass as well as the uniformity of the biomass powder.

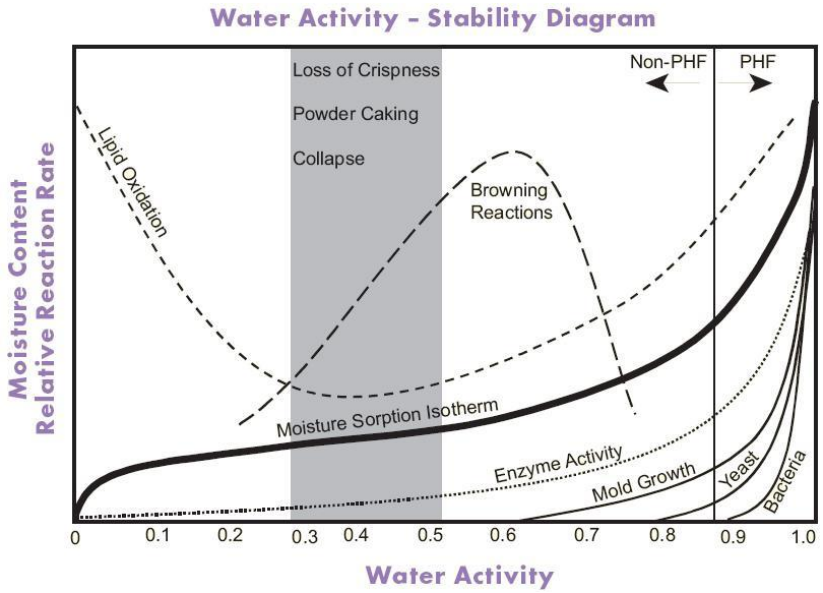

Figure 10. Water activity diagram, showing minimum lipid oxidation for water activity at 0.3 - 0.5 (Aqua Lab, 2012).

Water activity in the dried material is an important factor regarding oxidation of lipids. As can be seen in the chart, lipid oxidation is lowest with water activity at 0.3 to 0.5. To achieve this range of water activity the moisture in the dried material should be around 4% to 6%.

Freeze drying

Freeze drying, also known as Lyophilize, is an expensive method that is widely used to dry microalgae on lab scale. Freeze drying is not suitable for large scale commercial recovery of microalgae products due to high expense (Molina Grima et al., 2003). Freeze drying can be divided into four main operations: freezing, vacuum, sublimation and condensing (Ratti, 2001). First there is freezing of the material. By freezing the material slowly it will form larger intracellular ice crystals, the ice crystal size influences cell disruption efficiency (Chisti & Moo-Young, 1986) and drying speed. Larger ice crystals have better cell disruption efficiency. The frozen material is subjected to heat energy at a pressure below 4.6 mmHg which is the pressure for the triple point of water, shown in

Figure *11*. Under those conditions the ice sublimes to vapor, about 2800 kJ of heat are required to remove 1 kg of ice (Chisti & Moo-young, 1999). Ice crystals leave cavities after sublimation in the lyophilized dry material. Big ice crystals leave big cavities which make drying speed faster but small ice crystals leave smaller cavities and therefore reduce drying speed.

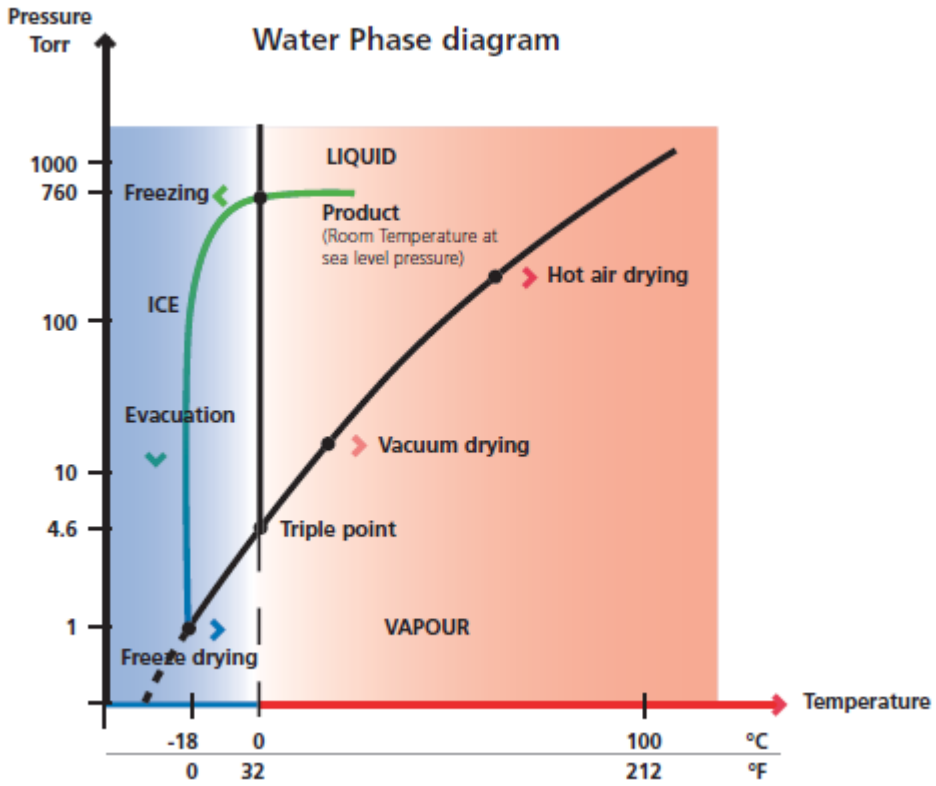

Figure 11. Three phase water diagram showing the freeze drying process (GEA Niro, 2013).

The drying consists of two stages; primary stage is to remove frozen water, reduce moisture to below 20% w/w and secondary drying removes sorbed water, reduce moisture to the required value (often below 1% w/w). Energy consumption breakdown for the four main operations is shown in Figure 12.

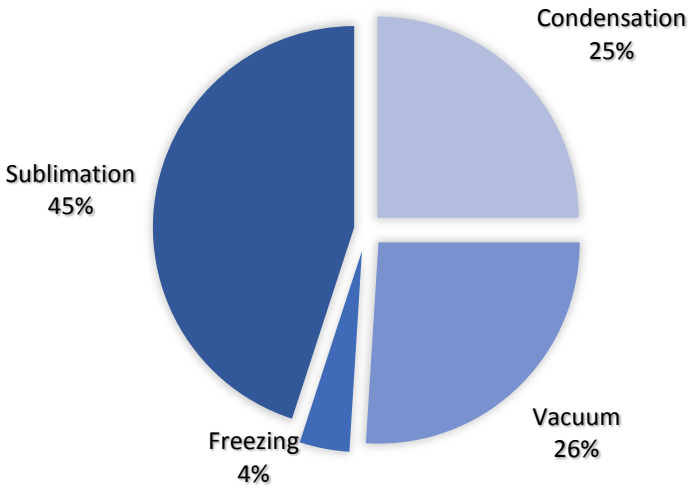

Figure 12. Energy consumption breakdown for freeze drying based on Ratti (2001).

Spray drying

When producing high value products (>$1,000 ton^{-1}) spray drying can be adequate. Spray drying is costly and the heat can cause deterioration of some valuable components such as pigments (Molina Grima et al., 2003). Spray drying consists for four basic steps: Atomization, drying, particle formation and powder recovery. The liquid feed is atomized either through a nozzle with pressure or compressed gas or through a rotary atomizer which rotates at high speed. The atomizer turns the liquid feed to droplets. The atomized feed is dried with hot air or nitrogen through a gas disperser. The deciding parameters for the drying process are temperature, flow rate and droplet size. All critical process parameters are kept constant throughout the batch. Evaporation starts immediately when the hot process gas comes in contact with the droplet. The thermal energy is consumed by the evaporation keeping the droplets temperature at harmless level, making spray drying suitable for heat sensitive products. When the liquid from the particle has evaporated it falls to the bottom of the chamber. The powder is recovered from the exhaust gases with a cyclone or a bag filter (GEA, 2012).

3.1.4 Extraction

Solvent extraction

When using solvent extraction it is necessary to choose the solvent wisely. The solvent should be volatile for easy removal, free from toxic and/or reactive impurities, able to form a two-phase system with water (remove non-lipids) and be unable to extract undesirable compounds (Molina Grima, González, & Giménez, 2013). Important properties and prices of two solvents, hexane and ethanol, are presented in Table 9.

Table 9. Important properties of hexane and ethanol as solvents.

Solvent	Boiling point (°C)	Density (gr/cm^3)	$/200 L barrel*	$/L*
Hexane	68.5	0.68	500	2.5
Ethanol	78.4	0.81	565	2.8

* Price in Iceland without tax from Olís ehf

Accelerated solvent extraction

Accelerated solvent extraction (ASE) is when solvent extraction is enhanced by using organic solvents at temperatures and pressures above their boiling point. It is possible to use ASE on solid and semi-solid matrices, the biomass must be dried prior to extraction (Cooney, Young, & Nagle, 2009).

Supercritical CO_2

Supercritical fluid extraction (SCF) produce highly purified extracts and is safe for thermally sensitive products (Mendes, Nobre, Cardoso, Pereira, & Palavra, 2003). SCF is based upon the enhanced solvating power of fluids when raised above their critical point. SFE uses chemicals which can behave both as a liquid and gas. It is the combination of liquid and gas properties which makes the diffusion coefficients better than liquids, liquid state has solvating properties and gas state has mass transfer properties. Carbon dioxide is often chosen because of its critical properties, 31.1 °C and pressure of 72.9 ATM, chemical inertness and the fact that compounds can be obtained without toxic organic solvents (Cooney et al., 2009). By using CO_2 waste from industry it is possible to counteract greenhouse gas effects. Dry material is placed in a cell where the temperature, pressure, extraction time and solvent flow rate is adjustable. These four factors affect the extraction efficiency. The supercritical fluid, which extracts the lipids, is inserted to the cell in a gas state. The temperature and pressure is set according to critical properties of the chemical, to achieve liquid state. When the extraction process is complete it is easy to separate the extract from the solvent by changing the temperature and pressure again to atmospheric conditions which will transform the solvent back to a gaseous state. Carbon dioxide is the most popular solvent but other fluids have been used; ethane, water, methanol, ethane, nitrous oxide, sulfur hexafluoride, n-butane and pentane (Herrero, Cifuentes, & Ibañez, 2006). It is possible to use a co-solvent with supercritical fluids. By using ethanol as a co-solvent it is possible the increase the polarity of the extraction solvent and alter the viscosity of the fluid, this will increase the solvating power of CO_2 as well as decreasing the required extraction temperature and pressure which results in better efficiency (Cooney et al., 2009).

3.2 Recent work

3.2.1 Harvesting

Gravity sedimentation

Danquah et al. (2009) found that microalgae settling by gravity in a glass container settled with different velocities according to light conditions at the settling destination and growth rate at which they were harvested. This is because the microalgae cells are actively photosynthesizing with a high metabolism rate and unicellular mobility in the presence of daylight and/or high growth rate phase. When photosynthesizing the microalgae cells hinder aggregation and reduces settling rate. Microalgae cells exposed to darkness and/or low growth rate phase do not photosynthesize and the cells tend to aggregate and settle faster.

Flocculation

Chidambara Murthy (2005) studied flocculation of *D.salina* with $Al_2(SO_4)_3$ in the concentration 0.2, 0.4, 0.6, 0.8, 1.0 and 2.0 mM, $FeCl_3$ in the concentration 0.1, 0.2 up to 0.5, 0.75 and 1.0 mM, chitosan in the concentration 0.01, 0.02, 0.03, 0.04, 0.05 and 0.10 mg L^{-1} and altering the pH. Of these flocculation methods the $Al_2(SO_4)_3$ in the concentration of 0.4 and 0.6 mM, and by altering the pH to 10.5 achieved the most flocculation in 60 min.

Horiuchi et al. (2003) effectively flocculated the algae *Dunaliella tertiolecta* by controlling the pH and maintaining it at 8.6 to 10.5 using NaOH. More than 90% flocculation efficiency was achieved at pH of 10.1.

Filtration

Danquah et al. (2009) experimented with cross flow filtration on the microalgae strain *Tetraselmis Suecica*. Cross flow filtration of 20 L high growth rate phase, 0.11 g/L day, with transmembrane pressure approximately 30 psi (207 kPa) for 25 min the culture concentrated 23 times by consuming 0.51 kWh/m^3 of supernatant removed. 20 L of low growth rate phase, 0.03 g/L day, with the same pressure and time the culture concentrated 48 times by consuming 0.38 kWh/m^3 of supernatant removed. According to this result it is more efficient to harvest from the culture when it is in a low growth rate phase.

3.2.2 Cell disruption

High pressure homogenizer

GEA Niro Inc experimented with cell disruption of *C.vulgaris*, the goal was to disrupt more than 80% of the cells. The homogenizer used was Panda NS1001L. The pressure applied was 1,000, 1,200 and 1,400 bar. Passing the suspension two times through 1,000 bar was also tried two times, when the suspension was cooled down before the second pass and when the suspension was not cooled before the second pass, shown in Table 10. A 96% disruption was achieved by passing the suspension once through 1,400 bar, shown in Figure 13 (GEA process engineering inc., 2013).

Table 10. Pressure values of high pressure homogenization on C.vulgaris cells (GEA process engineering inc., 2013).

Sample	Pressure (bar)	T_{inlet} °C	T_{outlet} °C	Cell disruption %
1	Not treated	-	-	Reference
2	1000	10	34	77%
3	1200	10	37	79%
4	1400	10	42	96%
5*	1000 x2	25 (before the second pass)	47	92%
6**	1000 x2	38 (before the second pass)	55	86%

* The cell suspension was cooled before the second pass

** The cells suspension was not cooled before the second pass

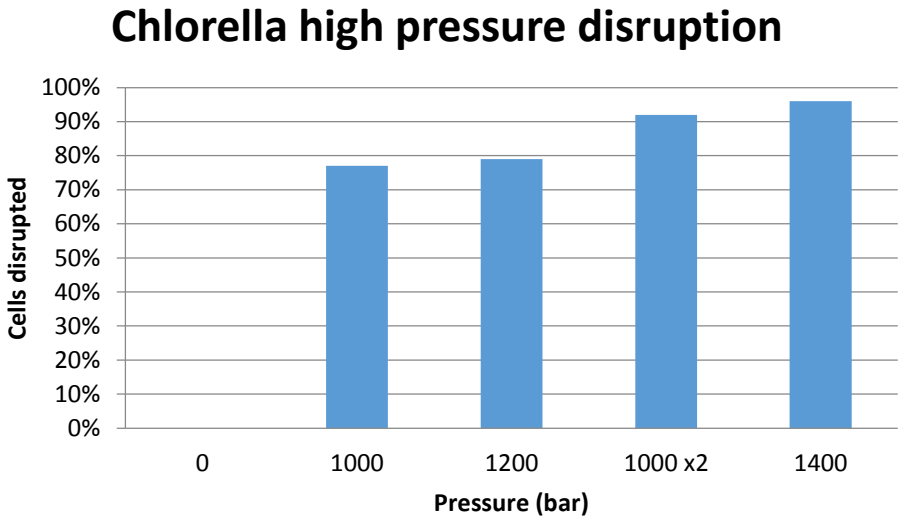

Figure 13. Percentage of C.vulgaris cells disrupted under various pressures (GEA process engineering inc., 2013).

Figure 13 shows the percent of surviving cells according to the pressure applied. As can be seen only 4% of cells survived the 1400 bar pressure.

3.2.3 Drying

Ryckebosh et al. (2011) studied the influence of short term storage and spray- and freeze drying on the stability of lipids and carotenoids from the microalgae *Phaeodactylum tricornutum (P.tricornutum)*. The storage time was 14 and 35 days, packaging was either vacuum packed or not vacuum packed and storage temperature was -20 °C, 4 °C and 20 °C. When the microalgae had been freeze dried it was more vulnerable to lipolysis upon storage than spray dried but when spray dried it was more vulnerable to oxidation. Short storage of fresh microalgae paste had lower lipid content due to susceptibility to

pronounced lipolysis. Effects of storage on freeze dried, spray dried and fresh *P.tricornutum* is shown in Figure 14 and Figure 15.

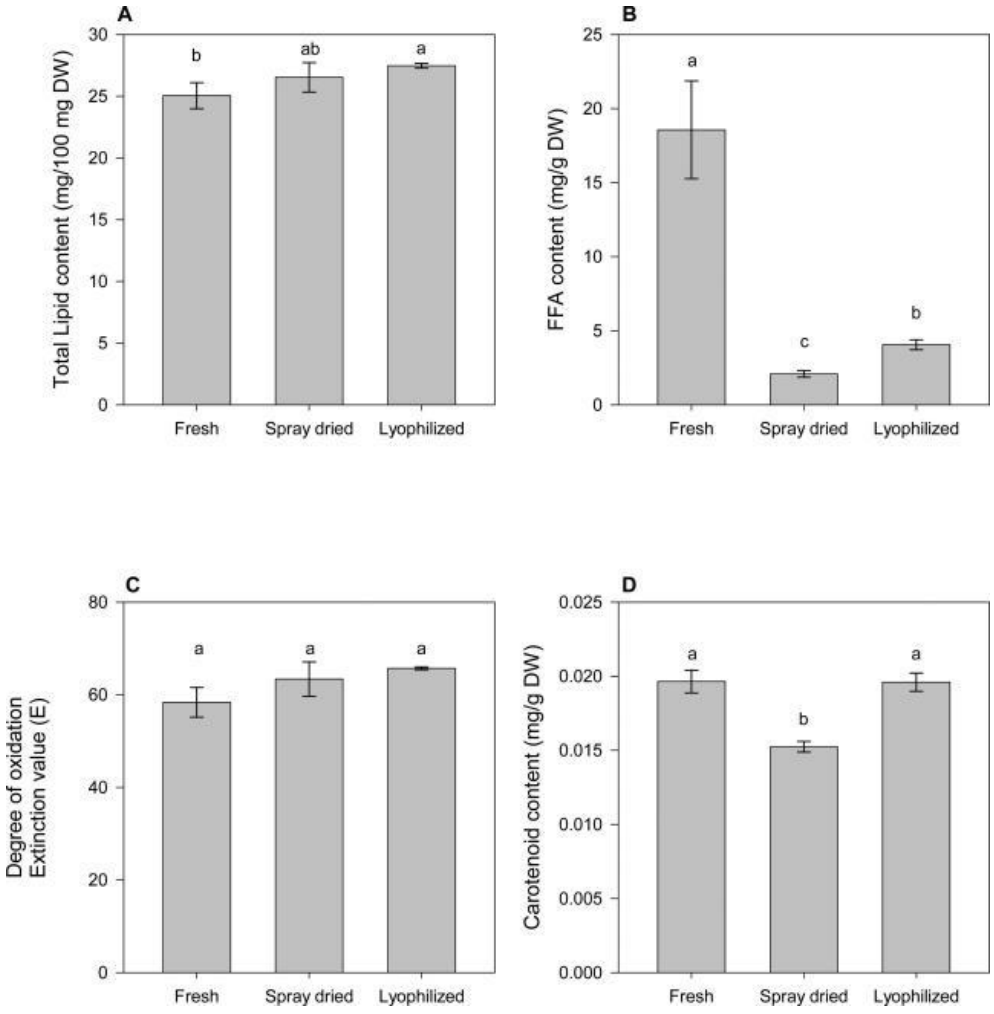

Figure 14. Influence of short term storage, spray drying and freeze drying of P.tricornutum on the total lipid content (A), Free Fatty Acid content (B), degree of oxidation (C), and carotenoid content (D) (Ryckebosch, Muylaert, Eeckhout, Ruyssen, & Foubert, 2011)

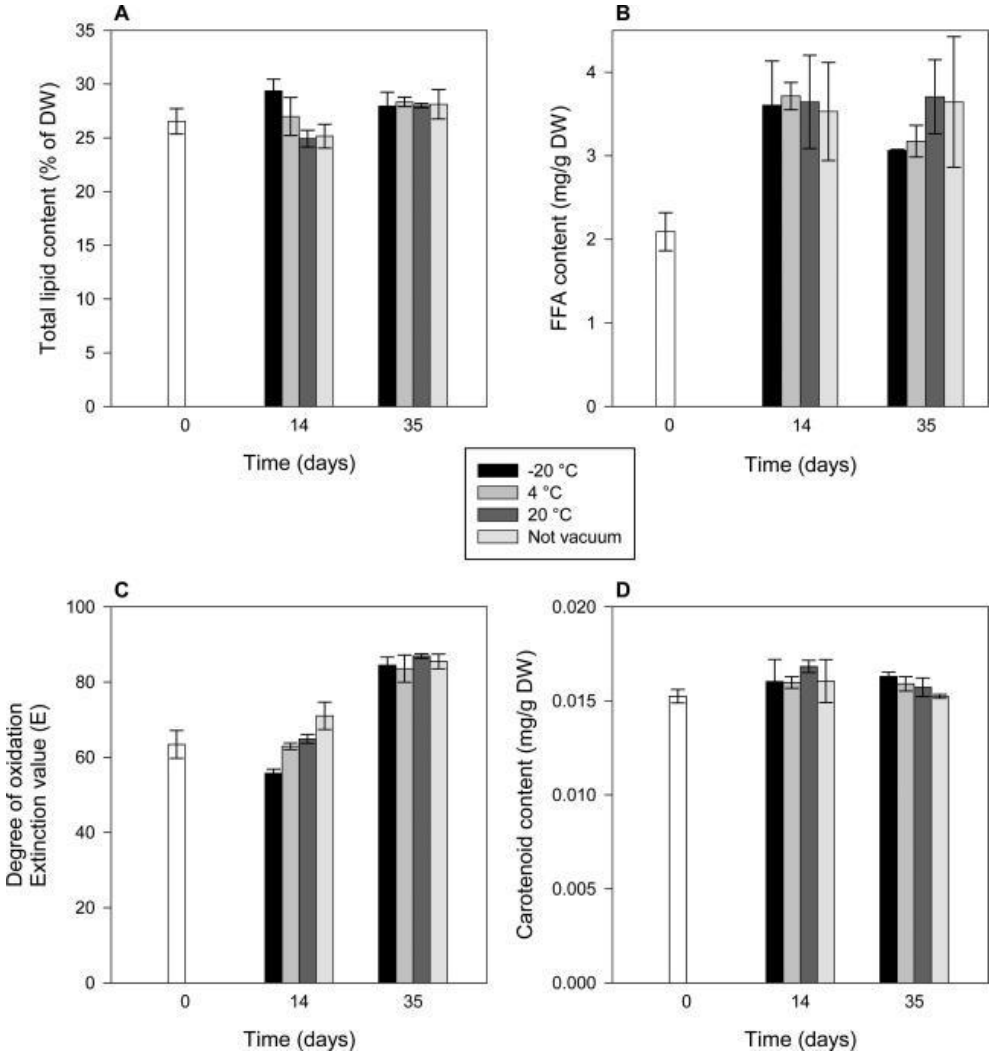

Figure 15. Influence of storage time and conditions on the total lipid content (A), FFA content (B), degree of oxidation (C), and carotenoid content (D) of spray dried P. tricornutum (Ryckebosch, Muylaert, Eeckhout, Ruyssen, & Foubert, 2011).

Chidambara Murthy (2005) spray dried *D.salina* where the feed was 15%-20% solids and the feed rate was 6 L hour^{-1}. The inside temperature was at 160±5 °C and the outlet temperature was at 80±5 °C. The composition of the spray dried powder is showed in Table 11.

Table 11. Composition of D.salina dry weight (Chidambara Murthy, 2005).

Parameter	Dry weight (%)
Protein	19±1.2
Lipid	7.2±0.35
Carotenoids	1.7±0.01
Carbohydrate	24.5±1.1
Moisture	2.1±0.2
Total ash	43.3±2.0

Leach et al. (1998) experimented with spray drying *D.salina* to β-carotene rich powder. They investigated three varying factors; inlet temperature (°C), outlet temperature (°C) and the solids content of the feed to the dryer (% w/w) on β-carotene recovery. Carotenoid recovery yielded higher with lower outlet temperature, β-carotene recovery varying from 57% and 91%. When biomass was microencapsulated it yielded 100% recoveries. Microencapsulation significantly increased the storage stability but non-microencapsulated powders showed 90% degradation over a 7-day period in presence of natural light and oxygen. The feed that was encapsulated was mixed with a polymer mixture of maltodextrin dextrose equivalent 12 and gum arabic, reducing the carotenoid as a percantage of solids. The results of the experiment are prestended in Table 12.

Table 12. Effect of spray drying conditions on β-carotene recoveries in D.salina biomass (Leach, Oliveira, & Morais, 1998).

Feed solids (% w/v)	T_{inlet} (°C)	T_{outlet}(°C)	Powder solids (% w/w)	β-carotene (% w/w solid)	β-carotene recovery (%)	β-carotene in starting material (% w/w)
4.50	200	110	95.85 ± 0.98	0.62 ± 0.02	90.89	0.68
		120	98.26 ± 1.32	0.79 ± 0.04	76.23	1.04
	265	110	95.28 ± 0.92	0.56 ± 0.05	81.12	0.69
		120	95.06 ± 0.30	0.47 ± 0.02	81.36	0.58
8.75	200	110	94.872 ± 0.46	0.24 ± 0.01	67.54	0.36
		120	94.55 ± 0.18	0.16 ± 0.02	56.92	0.28
	265	110	95.84 ± 0.54	0.27 ± 0.01	90.33	0.30
		120	94.61 ± 0.15	0.34 ± 0.02	82.93	0.40
15.43*	200	120	98.83 ± 0.17	0.21 ± 0.01	100.00	0.21
	265	120	98.15 ± 0.20	0.22 ± 0.01	100.00	0.22

* Microencapsulated

Orset et al. (1999) experimented with spray drying *D.salina* biomass with addition of antioxidants. There was a minimal loss of β-carotene and isomerization during processing without exogenous antioxidants as well as processing with butylated hydroxytoluene (BHT) and tert-butylhydroquinone (TBHQ). By adding TBHQ the degradation was significally minimized. Addition of tocopherol-based antioxidants in the drying process resulted in degradation of 52-72% of β-carotene. Of the antioxidants studied in this report only TBHQ significantly minimized degradiation. Light and oxigen also induce degradation and by restricting light and flushing with nitrogen to exclude oxygen degradation can be reduced.

3.2.4 Extraction

Experiments with the main extraction methods for microalgae have been gathered by Fernández-Sevilla et al. (2010) and are listed in Table 13.

Table 13. Selected carotenoid recovery methods gathered by Fernández-Sevilla et al. (2010).

Method	Description	Comment
Solvent extraction	ASE applied to dry biomass to extract antioxidants from *Spirulina platensis*	Pressurized high temperature (60-170 °C) extraction
	Hexane	4.3% at 170 °C, 15 min
	Ethanol	19.7% at 170 °C, 9 min
	Countercurrent L-L extraction of aqueous hydrolysate with hexane	Specifically for lutein. Recovery: 30 % in 1 step, 95 % in 6 countercurrent steps
Extraction with vegetable oils	Oil emulsion is formed with biomass slurry. Carotenoids are directly absorbed in oil droplets	80-95% carotenoid recovery claimed. Final 0.5-7.5% carotenoid weight in oil
SCF CO_2	Lutein from dry *Chlorella*, 50 MPa, 80 °C	Selective for lutein but much lower yield than Soxhlet
	Total carotenoids from *Nannochloropsis gaditana*	40% maximum yield at 60 °C, 400 bar. Poor chlorophyll separation
	Carotenoids from *D.salina*	50% maximum yield (compared to Dimethylformamide extraction) at 60 °C 300 bar
SCF CO_2 + cosolvent	5% molar ethanol tested on *Nannochloropsis gaditana*, *D.salina*	Moderate enhancement in recovery. Result is species-dependent

Chidambara Murthy (2005) extracted carotenoids from freeze dried biomass of *Dunaliella* with six different organic solvents: Acetone, chloroform, methanol, n-hexane, isopropyl alcohol and ethyl acetate. Isopropyl alcohol and n-hexane were used together in ration 1:1 in one experiment. Five volumes of solvent were used with a 5.0 mg sample. The solvent-sample mix had under gown a mortar with glass powder, mechanical grinding for 2-3 minutes and at last a sonication. Extractability of carotenoids in different solvents relative to the extraction of ethyl acetate is shown in Table 14. Total content of carotenoids in the biomass extracted with solvent was 22.0±2.0 mg 100 g^{-1}.

Table 14. Extractability of carotenoids in different solvents relative to the extraction of ethyl acetate (Chidambara Murthy, 2005).

Solvent used	Relative % extractability
Acetone	68.42
Chloroform	23.69
Methanol	31.57
n- hexane	65.78
n- hexane: IPA (1:1)	97.36
Ethyl acetate	100.00

Chidambara Murthy (2005) extracted carotenoids from freeze dried biomass of *Dunaliella* with eight different edible oils: Coconut, sesame, ground nut, mustard, olive, palm, rice bran, sun flower. The ratio was 1:5, five volumes of solvent. It was vortexed for 3 minutes, kept under low light for 2 to 3 hours and extracted with sonication for 4 to 5 minutes. The palm oil and olive oil extracted the most carotenoids, 4.846 mg 100 g^{-1} and 4,622 mg 100g^{-1} respectively, see Table 15. The maximum extraction from edible oil is 21.8 % of the maximum extracted from organic solvents.

Table 15. Amount of carotenoids extracted with different edible oils (Chidambara Murthy, 2005).

Oil	Amount of carotenoids extracted (mg 100 g^{-1})
Coconut	3.244
Sesame	2.948
Ground nut	3.110
Mustard	3.072
Olive	4.622
Palm	4.846
Rice bran	4.046
Sun flower	4.328

Herrero et al. (2006) optimized extraction of antioxidant compounds from *D.salina* by combining pressurized fluid extraction (PFE) and experimental design with different solvents; ethanol, hexane and water. Extraction temperature (40, 100 and 160 °C) and extraction time (5, 17.5, 30 min) were the main factors of the experiment. Extraction temperature had the strongest positive influence on extraction yield and antioxidant activity. The organic solvents showed better results than water, which provided the worst antioxidant activity and yield. Ethanol extracts showed the best yields, better than the other organic solvent Hexane. The highest yield, 34.6%, was obtained with ethanol at 160 °C for 30 minutes. Hexane extracts showed the best antioxidant activity but ethanol extracts could be considered actives (in absolute values). Antioxidant activity increased with increased temperature for all solvents but the yield was higher with higher temperature for water and ethanol but the best yields for hexane were obtained at medium temperature (100 °C). From this study it can be concluded that the chemical composition of *D.salina* is mainly based on medium-low-polarity compounds which explaines why ethanol yields the highest. Ethanol extracts was selected the most suitable of the solvents because of high yield, solvent safety (GRAS) and antioxidant activities, even though they are half of hexanes antioxidant activities. Also ethanol extract have different composition of carotenoids that can have a positive influence on the antioxidant activities.

Denery et al. (2004) experimented with pressurized fluid extraction (PFE) in natural product extracions. Aceton is used to extract carotenoids from *D.salina* in this study. This study showed that PFE, with 1,500 psi, has similar extraction yield as traditional extraction methods but used approximately half the amount of solvents and 70 minutes less per sample, from 90 minutes per sample to 20 minutes per sample. By optimizing the PFE conditions a complete extraction with minimum amount of solvent and time is possible as well as minimum chemical alteration of carotenoids. The comparison of extraction methods are presented in Table 16.

Table 16. PFE and traditional extraction of carotenoid compounds from D. salina (Denery, Dragull, Tang, & Li, 2004).

		Concentration of pigment[a]			
Solvent	**Target analyte**	**PFE**[b] (1 g)	**Traditional**[c] (1 g)	**PFE**[b] (25 g)	**Traditional**[c] (25 g)
Acetone	β-carotene (mg/g)	1.6 ± 0.1	1.4 ± 0.1	2.7 ± 0.3	2.3 ± 0.5
	Lutein (mg/g)	3.8 ± 0.7	4.5 ± 0.3	4.8 ± 1.0	4.3 ± 0.8
	Total pigments	50.3 ± 7.3	59.0 ± 4.7	61.7 ± 8.0	54.2 ± 7.1

[a] Average values of three replicates ± standard deviations

[b] PFE conditions: 1500 psi, two (for 1 g) and four (25 mg) 5 min extraction cycles

[c] Traditional extraction conditions: five to six 5 min sonication and centrifugation cycles

[d] Normalized absorbance values measured at 480 nm

Mendes et al. (2003) extracted β-carotene, mixture of *cis* (mainly 9-*cis*) and *trans* isomers, from *D.salina* with SCF CO_2. It‘s easier for the human body to absorb the *cis* isomers and thus it can be valuable to separating the two isomers. It is possible to separate isomers with SFE if there is significant difference in solubility of the compounds. The higher *cis/trans* ratio the better, since *cis* isomers are more easy absorbed by the human body. SCF CO_2 extraction on *D.salina* showed great improvement of the *cis/trans* ratio compared to traditional extraction with acetone. This study showed that the optimum conditions for SCF CO_2 regarding extraction yield for β-carotene is 300 bar and 40 °C.

3.3 Mass balance

The basis for mass balance is the law of conservation of mass, which states that mass can neither be created nor destroyed only changed.

$$m_{total\ in} = m_{total\ out}$$

Before making a mass balance it is necessary to declare the process classification (Felder & Rousseau, 2005), in this case the process is a batch process. The PBRs are drained every

48 hours and the drained suspension will go through the downstream process. Each period or run can be called a batch making it possible to wash the equipment between rounds.

Linear equation is used to determine the mass balance for evaporation and drying processes.

$$A_1 x + B_1 y = C_1$$
$$A_2 x + B_2 y = C_2$$

This can be presented in matrix form

$$\begin{pmatrix} A_1 & B_1 \\ A_2 & B_2 \end{pmatrix} \begin{pmatrix} x \\ y \end{pmatrix} = \begin{pmatrix} C_1 \\ C_2 \end{pmatrix}$$

The energy consumption for all pices of equipment in the downstream process will be accounted for and transformed to the form of kWh per unit volume or mass. When the process has been designed the total energy consumption can be calculated.

3.4 Cost analysis

When designing a downstream process the cost analysis gives a good idea whether the process is feasible. The total cost consists of the capital expenditure (CAPEX) and operating expenditure (OPEX). In this thesis a cost analysis will be made for several downstream process cases but the cost of culturing is not included since this thesis only covers the downstream process cost. Each downstream process case will be divided to the main process steps and other essential equipment. Units like piping and pumps which will be calculated as 25% of the total cost according to a rule of thumb when designing a process plant.

4 Algae Processing Experiments

Experiments were done on *D.salina* and *C.vulgaris*. Cell disruption was performed on *C.vulgaris*. The experiments made to disrupt the cell wall of *C.vulgaris* were on a lab scale. The main purpose of the experiments was to do a proof of concept that the methods tested could actually show some disruption, they are not optimized nor do they show similar efficiency as a industrial sized equipment specialized for microalgae cell disruption could. Harvesting experiment was performed on *D.salina* and the purpose was to proof that it would settle. Lipid content was analyzed on a freeze dried *D.salina* sample.

4.1 Harvesting

4.1.1 Centrifugation

A spin test was made on *D.salina* at GEA Westfalia in Iceland. Two samples of about 9 mL with the concentration of 0.25% TSS were centrifuged for 30 seconds and then for 3.5 minutes at 1000 *g*. The results are shown in Figure 16 and Figure 17. Settlement was achieved after 3.5 minutes as can be seen in Figure 17.

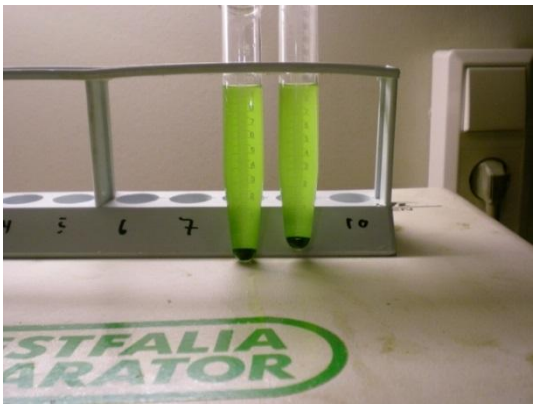

Figure 16. 9 mL sample of D.salina centrifuged for 30 seconds, there are still a lot of cells in the supernatant.

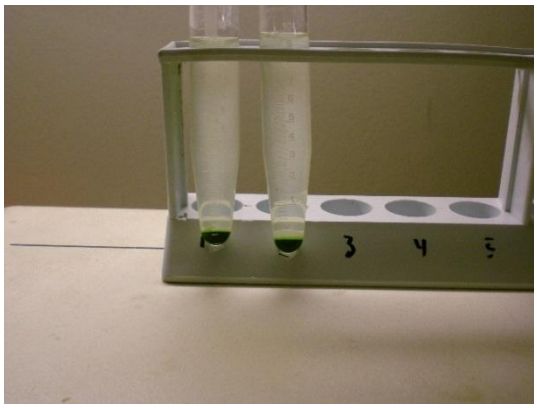

Figure 17. 9 mL sample of D.salina centrifuged for 3.5 minutes and visually the separation was effective.

4.1.2 Water content in paste

D.salina suspension with a concentration of about 0.25% was centrifuged at 4000 *g* for 5 minutes. The supernatant was carefully removed and the sediment was in form of paste. The paste was heated at 103 °C for 30 minutes in Sartorius MA 35 water content analyzer. The water content in the *D.salina* paste was 74.4%.

4.2 Cell disruption

4.2.1 Pulse Electric Field (PEF)

500 mL of *C.vulgaris* suspension with concentration of 1.045 % TSS was put into a pulse electric field with the goal to disrupt the cell wall. There were about 70 pulses per min and each pulse was 18 kV. The running time was 5 minutes. Temperature before the treatment was 22 °C and 35 °C after the treatment.

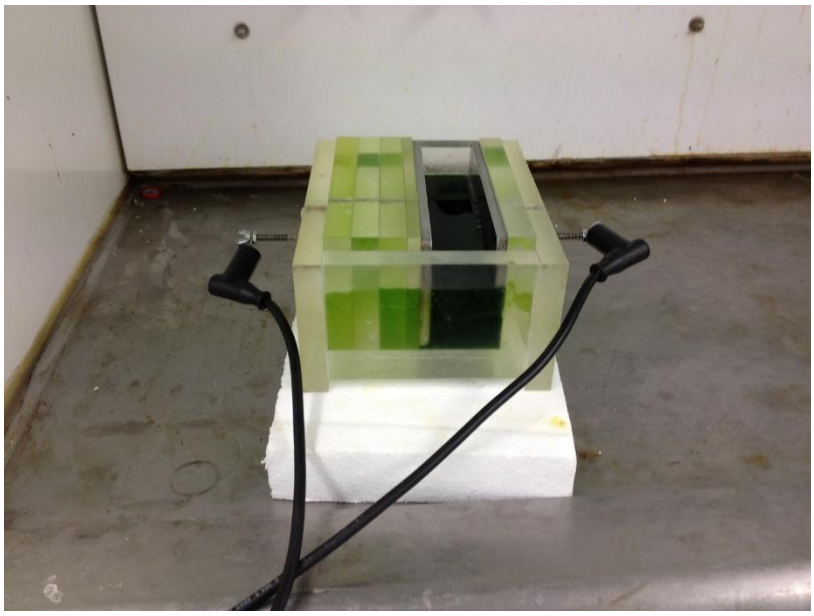

Figure 18. The suspension inside the PEF equipment.

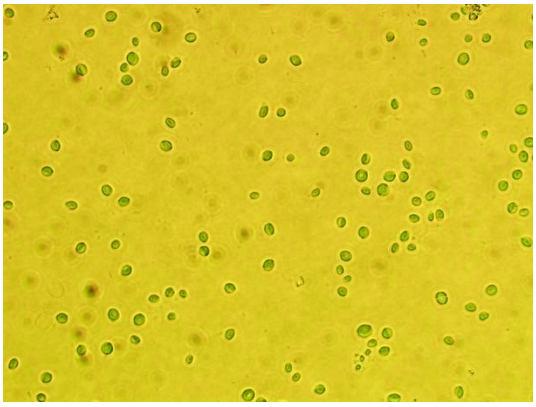

Figure 19. A microscopic view (100x) of the C.vulgaris sample before the treatment with pulse electric field.

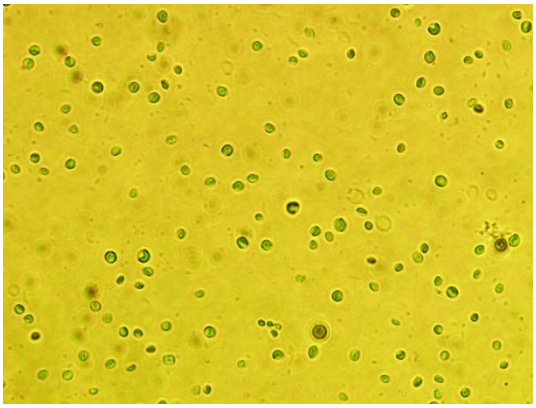

Figure 20. A microscopic view (100x) of the C.vulgaris sample after the treatment with pulse electric field.

As can be seen in Figure 19 and Figure 20 the PEF treatment did not achieve visual cell disruption.

4.2.2 Homogenizer

The smaller one

300 mL of *C.vulgaris* suspension with concentration of 1.045 % TSS was put into a high pressure homogenizer shown in Figure 21. The pressure was raised to 4.5 bar pressure and suddenly back to atmospheric pressure, 1 bar. The pressure difference was supposed to disrupt the cell.

Figure 21. The high pressure homogenizer used in the experiment.

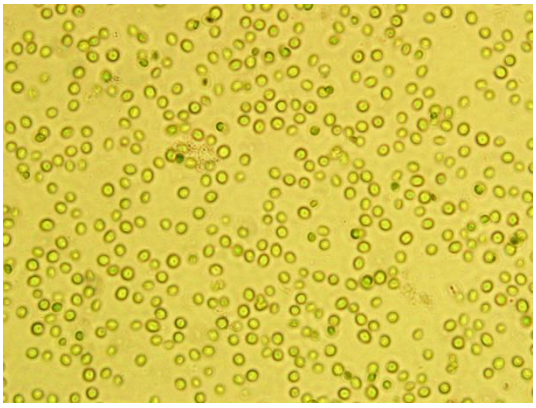

Figure 22. A microscopic view (100x) of the C.vulgaris sample before the treatment with high pressure homogenizer.

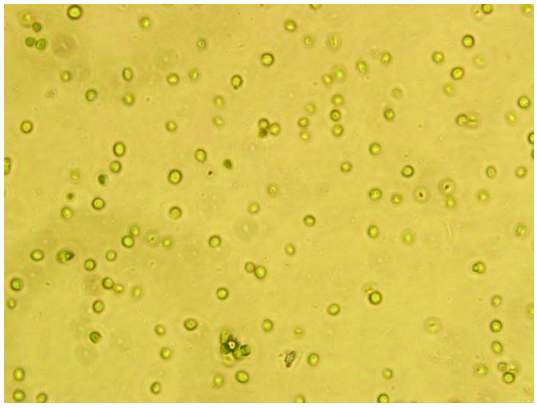

Figure 23. A microscopic view (100x) of the C.vulgaris sample after the treatment with high pressure homogenizer.

As can be seen in Figure 22 and Figure 23 there are visually fewer cells after the treatment with the high pressure homogenizer which indicates that there has been some disruption.

The larger one

A 300 mL sample of *C.vulgaris* suspension with concentration of 1.045 % TSS was put into the high pressure homogenizer shown in Figure 24. The pressure was raised to about 137 bar pressure and suddenly back to atmospheric pressure. This pressure difference was supposed to disrupt the cell.

Figure 24. The high pressure homogenizer used in the experiment.

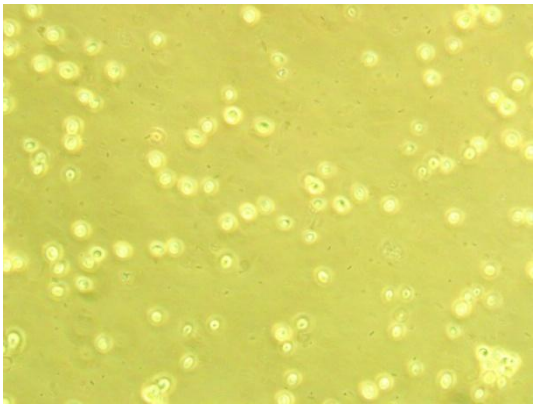

Figure 25. A microscopic view (100x) of the C.vulgaris sample before the treatment with a high pressure homogenizer.

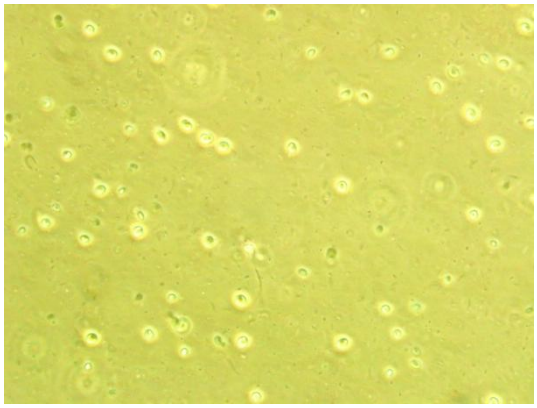

Figure 26. A microscopic view (100x) of the C.vulgaris sample after the treatment with a high pressure homogenizer.

As can be seen in Figure 25 and Figure 26 there seem to be fewer cells after the treatment. The cell density difference is not that much but seems to be slightly less after the treatment.

4.2.3 French Press

A 30 mL sample of *C.vulgaris* with concentration of 1.045 % TSS was put into a french press with pressure of 62 bar shown in Figure 27. The sample was put three times through the french press under the same pressure.

Figure 27. The French press used in the experiment.

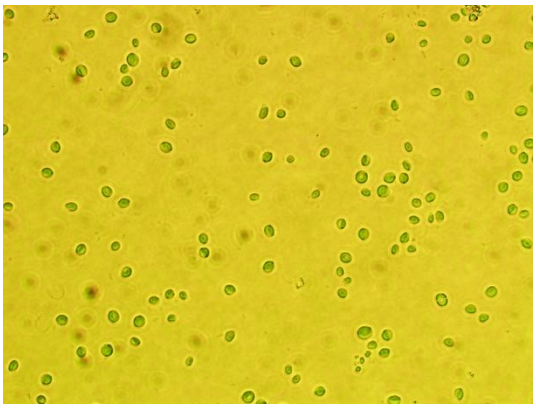

Figure 28. A microscopic view (100x) of the C.vulgaris sample before the treatment in french press with pressure of 62 bar.

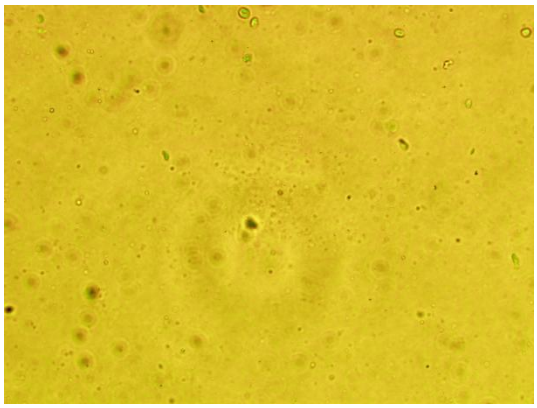

Figure 29. A microscopic view (100x) of the C.vulgaris sample after the treatment in french press with pressure of 62 bar.

As can be seen in Figure 28 and Figure 29 there has clearly been a massive cell disruption. The french press showed by far the most success in disruption the cellular wall of all the experiments performed.

4.3 Lipid analysis

A sample of *D.salina* paste with concentration of 24.4% TSS was freeze dried for 4 days. The lipid amount was analyzed from the freeze dried sample showing lipid content of 23.6%.

5 The Selection of Downstream Equipment

Certain assumption about the size and operating procedures of the PBR has to be made. The following assumptions are made for the calculations:

- The production is close to a power plant.
    - Inexpensive electricity is available from the power plant.
    - The power plant is a source of CO_2 for the cultivation.
- The production is closed to a geothermal source.
- Dry weight of cell is 25% of fresh weight (Mandalam & Palsson, 1998).
- Density of the microalgae cells is the same as for water, for flow calculations. This is used for both *D.salina* and *C.vulgaris*.
    - Density of fresh *C.vulgaris* cell is 1.01 g/mL (Mandalam & Palsson, 1998).
- 50% of the plants volume is drained every 48 hours.
- *D.salina* paste water content analysis was 24.4% TSS after harvesting, for simplification in mass balance 25 % TSS will be used. For *D.salina* and *C.vulgaris*.
- Concentration of *D.salina* in suspension is 0.25% TSS, 2.5 grDW/L (This is according to optical density measures).
- Concentration of *C.vulgaris* in suspension is 1.045% TSS, 10.45 grDW/L (This is according to optical density measures).

The assumptions about the size of the PBR and its operating schedule are shown in Table 17 and Table 18.

Table 17. Assumptions regarding plant size and volume.

Plant Assumptions		
PBR/Module	33	unit
Modules in plant	27	unit
PBR Working Volume	22	L
Volume/Module	726	L/module
Volume/Plant	19.602	L/plant
Buffer tank for harvesting	10.000	L

Table 18. Assumptions regarding drainage of the PBR.

Drainage assumptions		
Drainage	50	%
Draining time/module	0,75	hours
Draining time/plant	20,25	hours
Drainage every (Period)	48	hours
Total flow from PBR	9801	L
Total flow from module to buffer tank	363	L
Flow rate from module to buffer tank	484	L/hour

The local energy available is geothermal steam and electricity. Since the downstream production is close to a power plant makes the travel distance for the electricity much shorter and therefore cheaper, how much the electricity will cost is unknown but in the cost analysis 1 kWh costs $0.08, it is highly likely that the electricity cost will be lower than that. The energy consumption for drying, evaporation and distillation is relatively high. It would be possible to reduce the energy cost by utilizing the geothermal steam for these pieces of equipment. The utilization of local energy sources is presented in Figure 30. A flow chart, Figure 33, presents various possibilities of processing microalgae according to different culturing conditions and cellular structure.

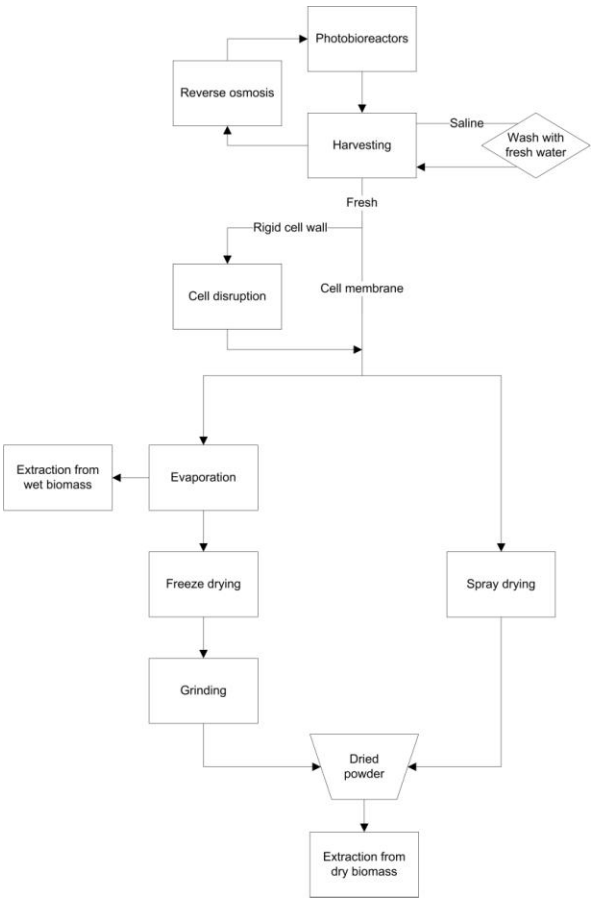

Figure 33. Flow chart showing various ways for a downstream process.

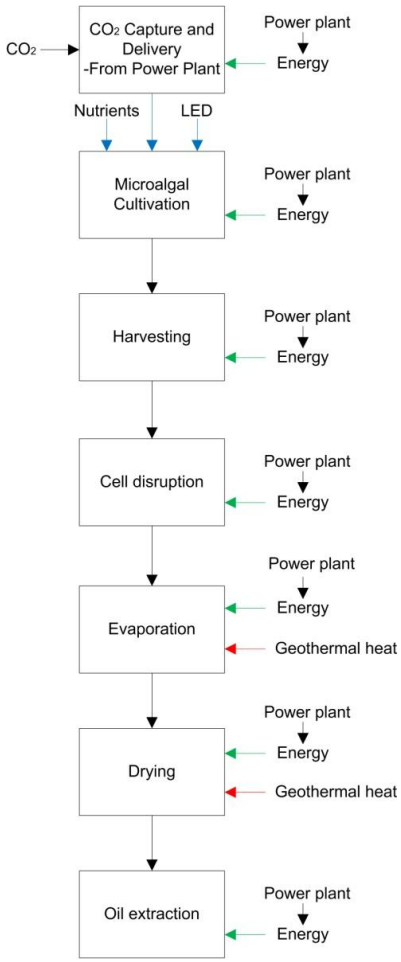

Figure 30. A simple flow chart of the downstream process showing where it is possible to utilize local energy.

5.1.1 Harvesting

The harvesting method will have to harvest microalgae from 9.801 L of suspension. It depends on the microalgae cultured what the concentration is but in this thesis the cases are *D.salina* with a concentration of 0.25% TSS and *C.vulgaris* with a concentration of 1.045% TSS. Since the goal is to produce a product of high value it is feasible to have as much control over the process as possible, less control can lead to the risk of a product with less quality. According to the comparison in Table 20, centrifugation is the most reliable method in these experiments with ability to achieve concentration of 22% TSS with reliability over 90%. Cross flow filtration can achieve higher concentration but with larger variations in TSS after harvesting and less reliability in recovery. The advantages and disadvantages of the main harvesting methods are presented in Table 19.

Table 19. Advantages and disadvantages of the main harvesting methods (Rawat, Kumar, Mutanda, & Bux, 2012).

Method	Advantages	Disadvantages
Filtration	Low cost, water re-use	Slow, membrane fouling and clogging, limited volume, cell damage
Centrifugation	Rapid, easy, efficient	Very high energy input
Gravity sedimentation	Low cost, potential for water recycling	Slow, product deterioration, separation depends on cell density
Chemical flocculation	Low cost, low cell damage	Biomass toxicity, no water re-use, inefficient, potential to remove lipids, produces large quantity of sludge that increases the difficulty to dehydrate the biomass
Dissolved air flotation	Low cost, easy application at large scale	Needs flocculants, water re-use and product extraction may be negatively affected
Microstrainers	Easy operation, low cost construction, high filtration ratios	Strongly cell concentration dependant, smaller cells may undergo incomplete removal, difficulty and handling solids fluctuations
Cross flow filtration	Water re-use, removal of pathogen	Membrane fouling

Table 20. Comparison of mechanical harvesting methods for algae. Adapted from (Shelef, Sukenik, & Green, 1984), (Shen, Yuan, Pei, Wu, & Mao, 2009), (Greenwell, Laurens, Shields, Lovitt, & Flynn, 2010) and (Uduman, Qi, Danquah, Forde, & Hoadley, 2010)

Method	TSS after harvesting (%)	Recovery	Advantages	Disadvantages
Centrifugation	12 - 22	>90%	Reliable, high solids conc.	Energy intensive, High cost
Cross flow filtration	5 - 27	70 - 90%	Reliable, high solids conc.	Membrane fouling, high cost
Gravity sedimentation	0.5 - 3	10 - 90%	Low cost	Slow, unreliable
Dissolved air flotation	3 - 6	50 - 90%	Proven at large scale	Flocculants usually required

Despite the need for a higher initial investment and high ongoing costs for energy, labor and maintenance, because of the high volumes that must be treated and discharged, centrifugation using continuous flow and automatic discharge has been claimed to be one

of the most effective harvest methods for recovering *D.salina* (Tafreshi & Shariati, 2009). Mohn (1980) agrees that the centrifugation is a preferred method for recovering algae cells.

The suspension is fairly concentrated when compared to suspensions from open ponds which are normally much more dilute. Sayre (2009) claims that harvesting microalgae with flocculation or flotation is only marginally less expensive than harvesting by centrifugation. Chemical flocculants are not recommended for this process since the concentration is large enough to settle without them and the risk of contamination later in the process could decrease the value of some final products. The goal is to use everything made in the process, re-use the liquid separated in the harvesting method, defatted biomass and lipid. Nothing should be labeled as trash, rather as by-product which has certain value. By using chemical flocculation the usage of by-products might decrease because of the risk of contamination.

Flotation will be one of the methods recommended through OriginOils Algae Appliance equipment, there flotation is used to float the microalgae to the surface after it has been cell disrupted and flocculated with electromagnetic pulses. This method is less energy intensive than centrifugation. It also has the advantage of including a cell disruption method which would reduce the amount of equipment bought for the process, which is if the microalgae have a rigid cellular wall.

Washing

Since *D.salina* is cultured in saline environment it can be feasible to wash the salt away, it depends on the final product whether it is necessary. If the final step is to dry the microalgae it may be feasible to wash the salt away if the powder is meant for human consumption. If the goal is to extract lipid or fractionate pigments like β-carotene the washing step might not be necessary for that particular product but the by-product such as defatted biomass might have some restrictions on salt. The washing step consists of the addition of fresh water to the *D.salina* paste and back into the harvesting step.

Recommended equipment

1. GEA Clarifier SSE-06-22.
   It is possible to use the equipment both as clarifier and a purifier, which can be converted into a three phase separator in a short matter of time. The feed rate can get up to 1400 L/hour. It has and automatic discharger which is highly recommended due to the amount of suspension processed, this is the smallest automatic clarifier from GEA. If the suspension is highly saline the equipment should be flushed intensively with fresh water after each production stop. FOB price is about $50,000.

2. Evodos Type 25

   Evodos Type 25 is specially designed to harvest microalgae and uses a new technology called spiral plates technology. It is designed to automatically discharge the paste. It rotates at only 800 *g*'s making the discharge gentle and non-pressurized, which is suitable for shear sensitive products like *D.salina*. The energy demand is minimal making the heat difference between the feed and the discharge effluent flow <0.2 °C. The company claims that the separation efficiency and cut-off rates are well above the existing market norms. FOB price is about $200,000.
3. OriginOil – Algae Appliance model 200

   This equipment is specially designed for harvesting microalgae. It has got three features; harvesting by flotation, flocculation by electromagnetic pulse and cell disruption by electromagnetic pulse. OriginOil claim that 99% of bacteria will be killed using the equipment. The price could not be acquired but the FOB price for a smaller unit is about $50,000 so to give estimation for the larger unit it will be tripled in price making it $150,000.

5.1.2 Water re-use

Since half of the suspension is drained every 48 hours and only a few percent of that will go further than the harvesting step. When the microalgae have been harvested there are still some nutrients and in saline cases salt left in the water which would be suitable to re-use rather than pump away from the process. When using reverse osmosis to filter away the impurities it is possible to recycle some of the water and with further research on the impurities there might be found some valuables. Reverse osmosis is a good purification method.

5.1.3 Cell disruption

Mechanical disruption of the algal cell is generally considered preferable to chemical disruption as it avoids contamination and preserves the functionality of the cell content (Chisti & Moo-Young, 1986). Disadvantages of the mechanical methods include high energy requirements and generated heat with the disruption process. Therefore it is often necessary to add a cooling process which needs to be taken into consideration, valuable products are often heat sensitive. (Lee, Lewis, & Ashman, 2012). While disrupting cells lipids can leak into the medium and form a complex mixture which may increase the difficulty of separation (Harrison, 1991). Mechanical disruption is most effective and energy efficient when biomass concentrations of 100 to 200 g/L are used (Greenwell, Laurens, Shields, Lovitt, & Flynn, 2010)**.** The experiments made on *C.vulgaris* were done on a lab scale to try different disruption methods. The methods are not all available for industrial scale nor do they show optimized disruption efficiency.

Equipment options

1. Homogenizer from GEA Niro Soavi
   The method chosen for cell disruption is a high pressure homogenization, it was recommended by GEA for the disruption of Chlorella. It is capable of achieving high cell disruption efficiency (GEA process engineering inc., 2013), as can be seen in the recent work. The high energy consumption is a disadvantage but the fact that the plant has access to low cost electricity has a neutralizing effect on the disadvantage. It is possible to put pressure of 1,500 bar on the feed with flow rate of 50 L/hour. The flow rate will increase with lower pressure but the cell disruption might be reduced.

2. OriginOil Algae Appliance 200
   Same as before.

5.1.4 Evaporation

The evaporator will evaporate utilizing geothermal heat as well as having a pressure control to reduce the heat necessary to evaporate. The amount of biomass needing evaporation is 88.2 kg with concentration of 25% TSS. Evaporation will be used to dewater to 50% TSS. With the mass balance it is possible to calculate how many kg of vapour will have to be vaporized from the paste to go from 25% TSS to 50% TSS. Since some of the compounds are heat sensitive it is feasible to evaporate with low temperature, such as 25 °C. By using a steam properties table the difference between liquid enthalpy and enthalpy for saturated vapour is found, which is the enthalpy for steam. Than kJ kg^{-1} is changed to kWh kg^{-1} to calculate the total amount of kWh per the evaporation step. In Table 21 the values used from the steam table are shown.

Table 21. Properties from the steam properties table showing enthalpy change and kWh kg^{-1}.

Temperature(°C)	Vapour pressure (Bar)	$Enthalpy_{liquid}$ (kJ/kg)	$Enthalpy_{saturated\ vapour}$ (kJ/kg)	Δ Enthalpy (kJ/kg)	kWh/kg
25	3.2	104.9	2547.2	2442.3	0.7
70	31.19	292.98	2626.8	2333.8	0.6
100	101.4	419.0	2676.1	2257.1	0.6
150	475.8	632.2	2746.5	2114.3	0.6

Equipment options

1. Evaporator
   The evaporator would be specially built for the downstream process. There are companies in Iceland with the know how to build evaporators, i.e. Héðinn. It would be ideal to get a custom evaporator build with the advantage of utilizing geothermal heat, in the form of steam, to reduce energy consumption. An assumption is made that it is possible to evaporate at 25 °C and the energy consumption, kWh kg^{-1}, will be according to that from Table 21. According to an experienced guess from Sigurjón Arason it would be possible to build one for about $8,000.

2. Distillation
   The distillation equipment would be specially build for the downstream process. This equipment would be feasible to build in Iceland just as the evaporator in using the same utilization of geothermal steam. In this case the solvent chosen is hexane and to calculate the energy consumed the enthalpy difference between liquid and saturated vapour at 70 °C will be changed to kWh kg^{-1}, since the boiling point of hexane is just below 70 °C. This could also be used as the fractionation equipment when separating carotenoids from the lipid. According to an experienced guess from Sigurjón Arason it would be possible to build one for about $8,000.

3. Evaporator/distillation
   Using an evaporator and distillation equipment together has been done by Matís ltd. some years ago. By combining the two pieces of equipment it would be possible to use heat from the evaporator to distill either solvent from solvent-lipid mix or to fractionate smaller compounds from the lipid with solvent, such as pigments.

5.1.5 Drying

Only freeze drying and spray drying were reviewed in this thesis. Drum drying is less suitable due to longer contact with temperature risking more degradation of the heat sensitive compounds. Both freeze drying and spray drying will be used as a possibility for the downstream process. Freeze drying generally yields higher recovery of carotenoids than spray drying (Tafreshi & Shariati, 2009) but it is more time and energy consuming. There is a project at Matís ltd. aimed at designing a freeze dryer which utilizes geothermal heat. By utilizing geothermal heat the energy requirement will reduce, making the freeze dryer more suitable for large scale production than before. *D.salina* biomass is usually spray dried to produce algal powder (Borowitzka & Borowitzka, 1990). Spray dryers will sprinkle the biomass to droplets which demands the concentration of the biomass to be dilute enough for the atomizer to form droplets. On the other hand when using freeze drying the less water content in the biomass the better.

The freeze dryer will have to be able to dry 88.2 kg or 44.1 kg of *D.salina* biomass with concentration of 25% TSS or 50% TSS to 23.0 kg of powder with 96 % TSS depending on the pretreatment of the biomass. This is the total of 65.2 kg water evaporated or 21.1 kg water evaporated. Less energy is used when vaporizing water from the suspension than freeze drying it making it energy efficient to use an evaporator before the freeze dryer, up to a certain concentration point in this case 50% TSS. When the biomass has been freeze dried there is a large variety in particle size making a milling process necessary to standardize the particle size.

The spray dryer which is limited to lower concentration and will have to be able to dry 88.2 kg of *D.salina* biomass with the concentration of 25% TSS to 23.0 kg of powder with 96% TSS, with the total amount of 65.2 kg water evaporated every 48 hours. It has to be able to dry 368.7 kg of *C.vulgaris* biomass with the concentration of 25% TSS to 96.0 kg of powder with the concentration of 96% TSS, with the total amount of 272.7 kg water evaporated every 48 hours. The spray dryer uses about 1.9 kg steam per kg evaporated. To calculate the energy consumption the enthalpy difference for 150 °C in Table 21 is found and changed to kWh kg^{-1} which is than multiplied with 1.9. This adds up to the kWh per kg evaporated in the spray dryer.

Spray dried powder has got a standardized particle size making a treatment of grinding unnecessary. Each droplet is in contact with the drying steam for such a short time making it suitable for drying of heat sensitive compounds which pigments are.

When the microalgae has been dried it is more vulnerable to oxidation, oxidation will increase in light and oxygen. The spray dried biomass is more vulnerable to oxidation than freeze dried but since both are sensitive to oxidation it is necessary to make sure that the storage method is without light and the least amount of oxygen for both methods. If the powder is encapsulated that will work as a great storage method.

Chosen methods for the downstream process are both freeze drying and spray drying.

1. Freeze dryer – GEA Niro
   GEA Niro has got some options of freeze dryers but when in contact with GEA it was pointed out that most of the time freeze drying is only used for smaller scale. There were two types of freeze dryers mentioned, one with the ability to evaporate 55 kg of water during 8 hour batch and the other with the ability to evaporate 5 kg of water during 8 hour batch. The larger freeze dryer has the FOB price of about $450,000 and the smaller one has the FOB price of about $200,000.

2. Spray dryer – GEA Niro

   GEA Niro has got many options for spray dryers but the main focus was on how many kg of water it could evaporate per hour. There are other features to be decided later like the atomizer type which will control the particle size of the powder. There were two types of spray dryer recommended by GEA, larger and smaller. The larger one has the ability to evaporate 50 kg water per hour and has the FOB price of about $460,000 but the smaller one has the ability to evaporate 15 kg water per hour and has the FOB price of about $200,000.

3. Milling/Grinding

   When using freeze drying it is necessary to standardize the particle size. That is done with a milling machine, milling the larger particles down to a certain given size. According to an experienced guess from Sigurjón Arason the price is about $12,000.

5.1.6 Extraction

Solvent extraction

Solvent extraction is the extraction method with the best efficiency, better than mechanical extraction, vegetable oil and SCF CO_2, and works even better with cell disruption as a pretreatment. Hexane is a non-polar solvent which extracts non-polar lipids like β-carotene. When using solvents such as Hexane it is necessary to distill the solvent away from the lipid when the extraction has completed, this requires an extra energy input to the process. There is a risk of contamination from the solvent to the end product which can limit its use. The distilled solvent is recycled back to the solvent tank and used again. Cost is favorable for hexane use at this moment. There are certain concerns for hexane such as the availability, tighter emission restrictions, safety and its testing as a hazardous air pollutant. These factors have induced some interest in alternative solvents (Molina Grima, González, & Giménez, 2013). From the customers point of view the utilization of carotene/oil ratio is superior to the use of organic solvents, that is the customers are likely to look passed the use of an organic solvent if the carotene/oil ration is good (Tafreshi & Shariati, 2009). It is possible to use solvent extraction on wet biomass and dry biomass. Using a solvent extraction on dried biomass can increase the lipid yield, this is because of better percolation of the solvent in dried biomass than in wet biomass (Williams & Laurens, 2010). When using solvent extraction it is necessary to separate the defatted biomass from the lipid and solvent mixture. It depends on the extraction method if two-phase separation or three-phase separation is suitable. When using wet extraction there are three phases in the mixture: the lipid-solvent mixture, liquid and defatted biomass but when using extracting from dried biomass there are only the lipid-solvent mixture and the defatted biomass. In Table 22 the advantages and disadvantages of different extractions

methods are shown. To ensure that the solvent will come in touch with as much of the biomass as possible it is put through a screw conveyor before it is put into separation, this would be done for both wet extraction and extraction from dry biomass.

Table 22. Advantages and disadvantages of popular extraction methods for recovering oil from microalgae (Mercer & Armenta, 2011).

Extraction method	Advantages	Disadvantages
Pressing	1. Easy to use, no solvent involved	1. Large amount of sample required, slow process
Solvent extraction	1. Solvents used are relatively inexpensive; 2. Results are reproducible	1. Most organic solvents are highly flammable and/or toxic; solvent recovery is expensive and energy intensive; 2. Large volume of solvent is required
Supercritical fluid extraction	1. Non-toxic (no organic solvent residue in extracts); 2. 'Green solvent'; 3. Non-flammable and simple operation	1. High power consumption; 2. Expensive; expensive/difficult to scale up at this time
Ultrasonic assisted	1. Reduced extraction time; 2. Reduced solvent consumption; 3. Greater penetration of solvent into cellular materials; 4. Improved release of cell content into bulk medium	1. High power consumption; 2. Difficult to scale up

Supercritical CO_2

SCF CO_2 is a very interesting extraction method regarding recovery of pharmaceutical or nutraceutical substances because of its cleanness and the lack of toxicity generated from CO_2 as a solvent. The downside is that the extraction efficiency is not as good as classical solvent extraction. When using co-solvents to increase the extraction yield one of the main advantages of SCF CO_2 is spoiled, the easy separation of solvent and extract by changing the pressure and temperature. The equipment cost for SCF CO_2 extraction is higher than for classical solvent extraction (Fernández-Sevilla et al., 2010). A comparison between SCF CO_2 and traditional solvent extraction is shown in table 23.

Table 23. Comparison of solvent extraction and SCF CO2 made by (Mercer & Armenta, 2011).

Solvent extraction	SFC CO_2 extraction
Presence of solvent is inevitable; residual solvent concentration (usually in the order of ppm) depends on the solvent used	Procedure is completely free of solvents and thus extracts are very pure
Heavy metal contamination is also unavoidable, and depends on the solvent recycling procedure, source of raw material and what the machinery parts are made from	Free of heavy metals; not extracted, even if they're present in the raw material. There are no heavy metals present in CO_2 or the equipment
Inorganic salt content is also difficult to avoid (same reasons as above)	Free of inorganic salts (same reason as above)
Solvents have poor selectivity; during solvent removal, polar substances form polymers which lead to discoloration of the extract and poor flow characteristics	CO_2 is highly selective, so there is no change of polar substances forming polymers
Both polar and non-polar colors are extracted	Only non-polar colors get extracted
Solvent removal requires extra unit operations, which results in higher cost and lower recoveries	No extra unit operations required, and yield is very high

1. GEA Clarifier/purifier
   It depends on whether there are two or three phases to be separated. It is possible to change this clarifier to a purifier with a simple change. This is the same equipment as used in the harvesting step but by using it also in the extraction step it is possible to reduce the capital cost of buying two pieces of equipment.

2. Evodos Type 200
   The same rules apply for the usage of the Evodos Type 200, it is possible to change from two-phase separation to three-phase separation and use it in harvesting as well as for separation in extraction.

3. Screw Conveyor
   The screw conveyor is used to mix the solvent and microalgae powder or biomass together. This should get as much of the powder or biomass in contact with the solvent making the extraction more efficient. According to an experienced guess from Sigurjón Arason the price for a screw conveyor is about $4,000.

5.1.7 Tanks

Buffer tank

The drainage is 9801 L so the buffer tank has to have a volume of 10 m^3. Instead of buying one 10 m^3 tank it is recommended to buy two 5 m^3 tanks. The suspension can be

saline like in the case for Dunaliella salina so that has to be taken into consideration for the buffer tanks material. It would be recommended to have it made from 316 stainless steel. According to an experienced guess from Sigurjón Arason the price for one 5 m^3 tank made from 316 stainless steel is about $12,000, making the total cost for two tanks $24,000.

Fresh water tank

Since this tank is only meant for fresh water to use when washing saline microalgae paste it should be enough to have a 0.2 m^3 tank. According to an experienced guess from Sigurjón Arason the price for one 0.2 m^3 tank is about $2,000.

Solvent tank

According to the 5:1 ratio w/w of solvent versus powder it is necessary to have room for about 200 L of hexane, this will be a 0.2 m^3 tank. According to an experienced guess from Sigurjón Arason the price for one 0.2 m^3 tank is about $2,000.

Product tank - Powder

A small tank to store the powder made in the downstream process. According to an experienced guess from Sigurjón Arason the price for one 0.2 m^3 tank is about $2,000.

Product tank - Lipid

A small tank to store the lipid made in the downstream process. According to an experienced guess from Sigurjón Arason the price for one 0.2 m^3 tank is about $2,000.

5.2 Mass balance

There are four mass balance cases shown below which show the flow from the PBR to end product. The end products can be microalgae powder with 96% TSS, lipid, defatted biomass and carotenoids. It is assumed that all external liquid is gotten rid from the suspension in the harvesting/dewatering step and that only microalgae paste goes through, the water left is intracellular water. Another assumption is made regarding the harvesting step making the separation efficiency 90%, which means that 10% of the microalgae cells will go along with the external water. The lipid amount is 23.6% of dry weight according to the analysis made for *D.salina*.

The first downstream process proposition in Figure 31 includes spray drying and solvent extraction from 96% TSS powder. The final product is carotene rich lipid and the by-product defatted biomass. The mass balance states the amount of powder manufactured by drying 25% TSS to 96% TSS.

$$88.2\ kg = m_{Vapour} + m_{Powder}$$

$$75\% * 88.2\ kg = m_{Vapour} + 4\% * m_{Powder}$$

There is 88.2 kg of 25% TSS *D.salina* paste is dried to 96% TSS, making the water content 75% and 4% respectively. The result is that 65.2 kg of vapour and 23 kg of powder. The solvent ratio estimated for the process is 5:1 w/w, hexane to powder. That calculates to 114.8 kg of hexane and the density of hexane is 0.68 kg/L, corresponding to 169 L of hexane. The lipid amount is 23.6% in 100% TSS making it 22.7% in 96% TSS powder, corresponding to 5.2 kg of lipid from 23.0 kg of 96% TSS powder.

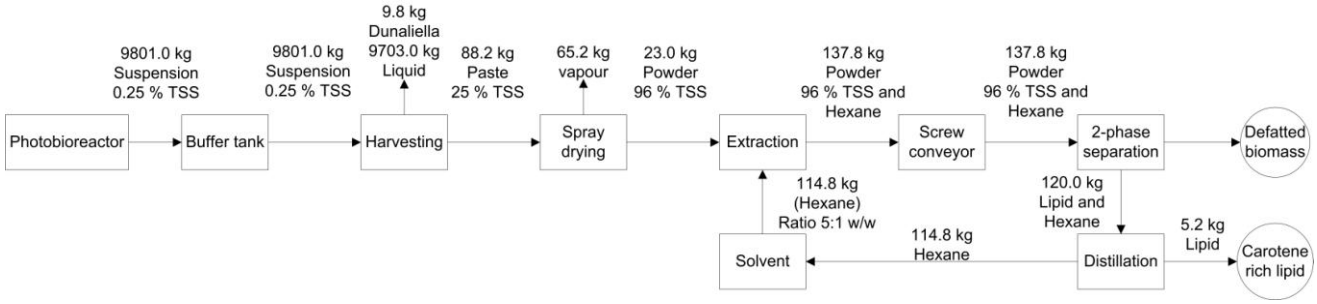

Figure 31. Mass balance for D.salina using spray drying and solvent extraction.

The second proposition in Figure 32 includes the same final products but uses freeze drying. By using freeze drying it might be suitable to add an evaporation step to concentrate the paste before the drying step. Freeze drying takes longer time and hasn't got standardized particle size, making a step of milling necessary.

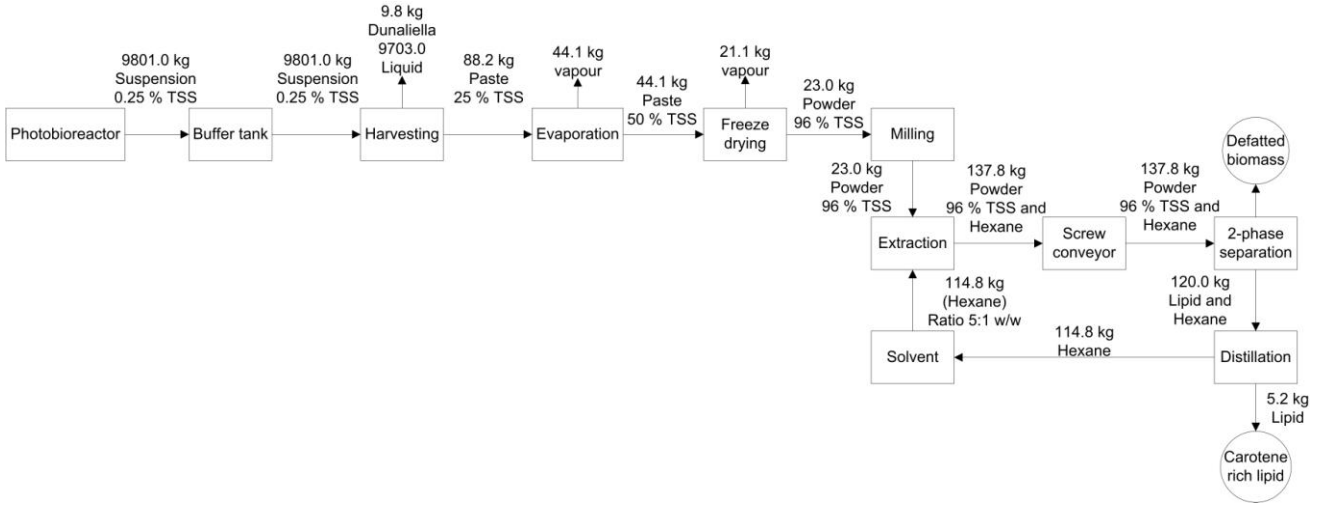

Figure 32. Mass balance for D.salina using freeze drying and solvent extraction.

The third proposition in Figure 33 for a *D.salina* downstream process indicates how pigments are extracted from microalgae. The mass balance stops at the carotene rich lipid

due to lack of information on the amount of carotenes and other pigments in the powder. The carotene rich lipid will go through a fractionation which will separate smaller compounds like pigments.

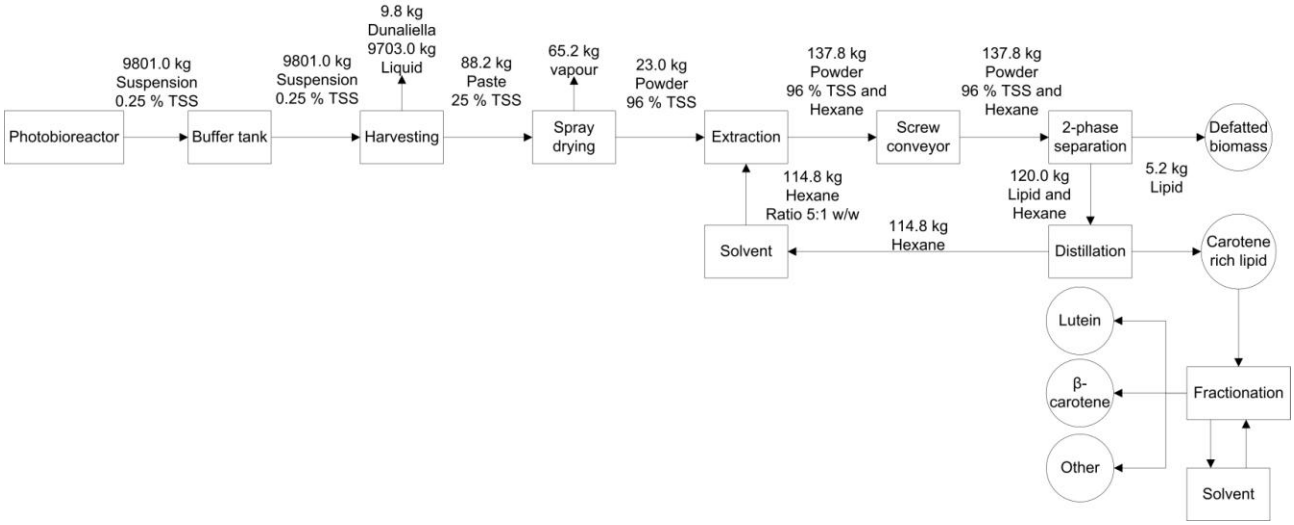

Figure 33. Mass balance for D.salina using spray drying and solvent extraction with the addition of fractionation to separate pigments from the lipid.

C.vulgaris downstream process is presented in Figure 34 where the final product is tablets. Since *C.vulgaris* has got a rigid cell wall a cell disruption step is included, there is no loss of material going through the step. The same principle is used to calculate the mass balance for the drying step as stated above.

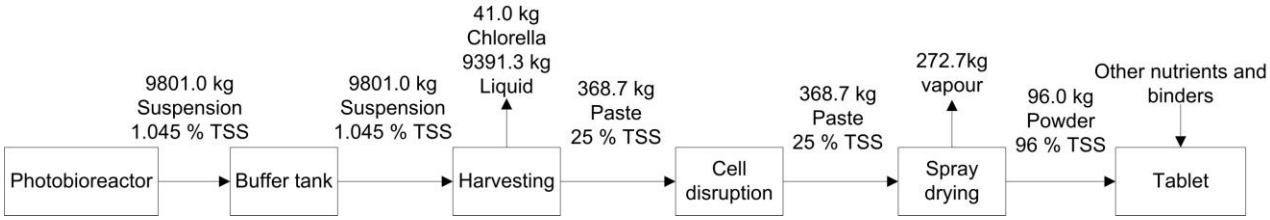

Figure 34. Mass balance for C.vulgaris using spray drying.

5.3 Cost analysis

The equipment possibilities for each step in the process are listed in Table 24 pointing out the capital cost, operating cost and energy requirement. The energy consumption wasn't available for all pieces of equipment so an estimation is made to calculate the OPEX. The OPEX is proportionally small compared to the CAPEX, therefore the estimation will not be a decisive factor for the cost analysis cases stated later. The energy consumption and CAPEX is calculated for harvesting, evaporating, drying and cell disruption. This makes it easy to input these important cost factors to the process designed, as can be seen in the cases later on. The cost analysis and cases are without labor cost.

Table 24. Cost analysis for the equipment mentioned as possibilities.

Equipment	CAPEX ($)	OPEX ($)/ year	kWh/year	Energy Requirements (kWh/m^3)	Energy Requirements (kWh/kg vapourized)
Harvesting					
GEA Clarifier*	51,000	294	3,650	2.0	-
Evodos Type 25	200,000	177	2,190	1.2	-
OriginOil AA model 200**	150,000	29	365	0.2	-
Harvesting tank ($5m^3$)	12,000	-	-	-	-
Fresh water tank	2,000	-	-	-	-
Cell disruption					
GEA Homogenizer	55,000	16	202	3.0	-
Evaporation					
Evaporator	8,000	440	5,461	-	0.7
Drying					-
Freeze dryer (55 kg /8 hour)	450,000	622	7,713	-	2.0
Spray dryer (15 kg/h)	200,000	1,071	13,286	-	1.1
Milling	12,000	-	-	-	-
Extraction					
GEA Purifier*	51,000	-	-	-	-
Evodos Type 25	200,000	-	-	-	-
Solvent tank	2,000	-	-	-	-
Distillation	8,000	1,096	13,587	-	0.6
Screw conveyor	4,000	-	-	-	-
Product storage					
Powder tank	2,000	-	-	-	-
Lipid tank	2,000	-	-	-	-

* The clarifier and the purifier are the same equipment, able to switch between operations.

** OriginOil is able to disrupt cells as well as harvest/dewater.

5.3.1 *D.salina* - Powder

In Table 25 and Table 26 are two cases for the production of *D.salina* powder. The CAPEX is by far the largest expenditure. The former case is less expensive because it is equipped with a spray dryer instead of a freeze dryer. With a freeze dryer it is necessary to buy more equipment as well as it being more time consuming and expensive.

Table 25. D.salina case 2 using spray drying, powder the final product.

Process	Pieces of equipment	Equipment	CAPEX ($)	OPEX($) / year	Total cost
Harvesting:	1	GEA Clarifier	51,000	294	
Tank:	2	Harvesting tank (5m3)	24,000	-	
Tank:	1	Fresh water tank	2,000	-	
Drying:	1	Spray dryer (15 kg/h)	200,000	1,071	
Product storage	1	Powder tank	2,000	-	
Pumps and pipes	1	25 % of total CAPEX	69,750	-	
Total cost			348,750	1,366	350,116

Table 26. D.salina case 2 using freeze drying, powder is the final product.

Process	Pieces of equipment	Equipment	CAPEX ($)	OPEX($) / year	Total cost
Harvesting:	1	GEA Clarifier	51,000	294	
Tank:	2	Harvesting tank (5m3)	24,000	-	
Tank:	1	Fresh water tank	2,000	-	
Evaporation:	1	Evaporator	8,000	440	
Drying:	1	Freeze dryer (55 kg /8 hour)	450,000	622	
Milling:	1	Milling	12,000	-	
Product storage	1	Powder tank	2,000	-	
Pumps and pipes	1	25 % of total CAPEX	137,250	-	
Total cost			686,250	1,357	687,607

5.3.2 *D.salina* - Carotene rich lipid

One case, Table 27, is presented for carotene rich lipid from *D.salina*. It is the same as case 1 for *D.salina* but with the addition of solvent extraction.

Table 27. D.salina case 3 using spray drying and solvent extraction, carotene rich lipid is the final product.

Process	Pieces of equipment	Equipment	CAPEX ($)	OPEX($) / year	Total cost
Harvesting:	1	GEA Clarifier	51,000	294	
Tank:	2	Harvesting tank (5m3)	24,000	-	
Tank:	1	Fresh water tank	2,000	-	
Drying:	1	Spray dryer (15 kg/h)	200,000	1,071	
Tank:	1	Solvent tank	2,000	-	
Mixing	1	Screw conveyor	4,000	-	
2-phase separation	1	GEA Clarifier	-	-	
Distillation	1	Distillation	8,000	1,096	
Product storage	1	Lipid tank	2,000	-	
Solvent	200L	Hexane	500	-	
Pumps and pipings	1	25 % of total CAPEX	73,250	-	
Total cost			366,750	2,462	369,212

5.3.3 *C.vulgaris* - Powder

Two cases are presented for *C.vulgaris*, Table 28 and Table 29, where the main difference is the harvesting method. The latter one has OriginOil AA model 200 for harvesting and cell disruption but the former has a clarifier for harvesting and a homogenizer for cell disruption.

Table 28. C.vulgaris case 1 using spray drying and a clarifier for harvesting and a homogenizer for cell disruption. Powder/tablet is the final product.

Process	Pieces of equipment	Equipment	CAPEX ($)	OPEX($) / year	Total cost
Harvesting:	1	GEA Clarifier	51,000	294	
Tank:	2	Harvesting tank (5m3)	24,000	-	
Cell disruption:	1	GEA Homogenizer	55,000	16	
Drying:	1	Spray dryer (15 kg/h)	200,000	4,479	
Product storage	1	Powder tank	2,000	-	
Pumps and pipes	1	25 % of total CAPEX	83,000	-	
Total cost			415,000	4,789	419,789

Table 29. C.vulgaris case 2 using spray drying and OriginOil AA model 200 for harvesting and cell disruption. Powder/tablet is the final product.

Process	Pieces of equipment	Equipment	CAPEX ($)	OPEX($) / year	Total cost
Harvesting:	1	OriginOil AA model 200**	150,000	29	
Tank:	2	Harvesting tank (5m3)	24,000	-	
Cell disruption:	1	OriginOil AA model 200**	-	-	
Drying:	1	Spray dryer (15 kg/h)	200,000	4,479	
Product storage	1	Powder tank	2,000	-	
Pumps and pipes	1	25 % of total CAPEX	94,000	-	
Total cost			470,000	4,508	474,508

5.3.4 Labor

It is estimated that one well educated and experienced chemist or chemical engineer should be enough to operate the downstream process.

5.3.5 Revenue

As can be seen under the chapter "Commercial products" the price for β-carotene products is on the range $275-$3,000 and the price for *C.vulgaris* health nutrition is $46. Since the isolation of carotenoids is not taken into consideration for the mass balance it may be assumed that the higher end of the β-carotene price is not available for the products in the *D.salina* cases. To make some assumptions the price for *D.salina* powder will be set to $300 and the *D.salina* lipid to $1,000. Since the price is a large variance a sensitivity analysis will be made to show the change in revenue according to the change in price for the powder. Since this is the theoretical possibility of revenue according to the given assumptions it is assumed that this production is running all year around, every 48 hours. The total number of batches is 182.5 per year. The total amount of final product can be found in the mass balance cases, in kg. The total cost of every case is shown in Table 30. The revenue according to final product is shown in Table 31.

Table 30. Total cost for each case stated.

Microalgae	Case	Product	Total cost ($)
D.salina	1	Powder	350,116
D.salina	2	Powder	687,607
D.salina	3	Lipid	369,212
C.vulgaris	1	Powder/tablets	419,789
C.vulgaris	2	Powder/tablets	474,508

Table 31. The revenue made from these three products according to given assumptions.

Product	Periods (batches/year)	Production (kg/year)	Price/kg	$/ Year
D.salina powder	182.5	4,198	300	1,259,250
D.salina lipid	182.5	950	1,000	949,694
C.vulgaris powder	182.5	17,523	46	806,081

5.3.6 Sensitivity analysis

Sensitivity analysis is made to show the difference in revenue/year according to price change. As can be seen in Table 32 and Figure 35 the difference from -40% to +40% is about $1,000,000.

Table 32. The correlation of revenue/year and change in price.

Factor	-40%	-30%	-20%	-10%	0%	10%	20%	30%	40%
Price ($)	180	210	240	270	300	330	360	390	420
Revenue/year (thousand $)	756	881	1,007	1,133	1,259	1,385	1,511	1,637	1,763

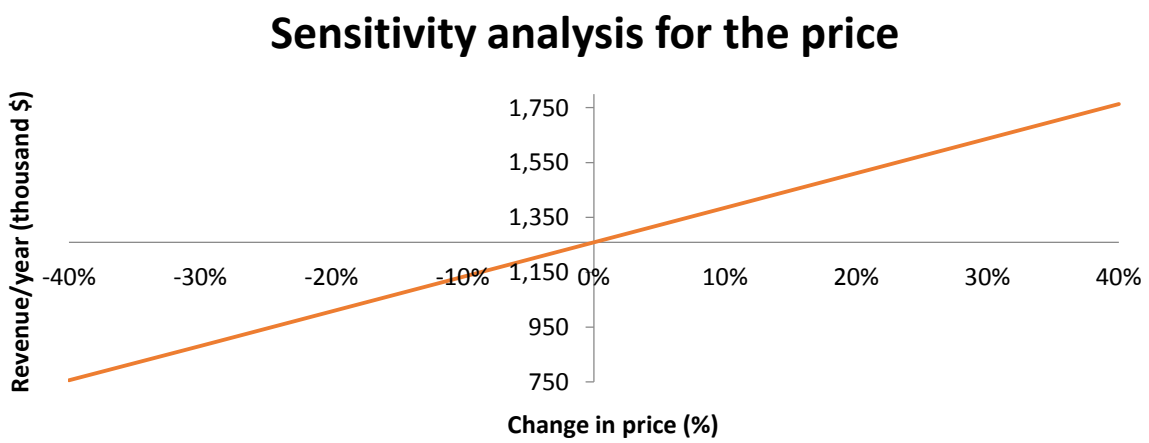

Figure 35. Sensitivity analysis for the price of D.salina powder.

6 Conclusion

This thesis is supposed to provide guidance for future designs and possibilities of a downstream process. There are a lot of factors to look into when the culture will evolve from lab scale to pilot scale. That will open up a lot of opportunities regarding testing and optimizing. The companies GEA and Alfa Laval have pilot scale process lines which they offer companies to test and optimize with their help and their equipment making it possible for startups to reduce the capital cost in research. The trend today is to utilize everything as much as possible and that means that it would be feasible to do further research on the by-products such as defatted biomass and the nutrients filtered away in the reverse osmosis. The defatted biomass is high in protein and might be a suitable product as such, i.e. one idea would be to study the market for beer foam which is made from protein products.

It would be good to have a flexible process line with connections which are easy to connect and disconnect, making it possible to produce multiple types of products by rearranging the equipment. SCF CO_2 is a very interesting possibility for the extraction of lipids and should be looked into in the future since it will most definitely continue to evolve making it less expensive.

At this time the methods recommended in the cases are the most suitable methods for high value product production. This industry is growing fast and new ideas, methods and pieces of equipment will see the light of day in the years to come hopefully making the process less energy consuming. Iceland has a great source of energy and with the right talent and set of mind it is very much possible to design a downstream process which will utilize local energy to valuable steps of the process like evaporation, drying and distillation. Hopefully the cost of electricity will still be economic.

According to the assumptions made regarding the revenue and cost analysis for the GeoChem project, it is certainly could be feasible to produce high value products from the culture. It should be noted that there are large cost factors missing like the cost of culturing, real estate, rent of building site and etc.

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