

# Future of algae based biodiesel production in the Netherlands



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Utrecht, July 2012

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## **Preface**

### *Context*

This thesis has been written during the course "Master thesis" (7.5 ECTS) within the master program Environmental Biology, track: Ecology & Natural Resource Management (E&NRM) at Utrecht University for the grade MSc in Life sciences.

### *Acknowledgements*

I would like to thank dr. J. N. M. Dekker for supervising me throughout the entire process and for the helpful comments on earlier versions of this thesis.

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## List of abbreviations

- EU - European Union
- EPA - eicosapentaenoic acid
- GHG - greenhouse gas
- GJ - giga joule =  $10^9$  Joule
- GWP - global warming potential
- ha - hectare
- l - litre
- LCA - life cycle analysis
- MCA - multi criteria analysis
- MJ - mega joule =  $10^6$  Joule
- Mt - million tonnes =  $10^9$  kg
- NER - net energy ratio
- NL - the Netherlands
- PAR - photoactive radiation
- PBR - photo-bioreactor
- SWOT - strengths weaknesses opportunities threats
- TJ - tera joule =  $10^{12}$  Joule
- TFC - total final consumption
- TPES - total primary energy supply
- U.S. - United States
- yr - year
- $\mu\text{m}$  - micrometer =  $10^{-6}$  meter
- $^{\circ}\text{C}$  - degree Celsius

## Summary

Microalgae are a promising feedstock for biodiesel production. To determine the future of biodiesel production from microalgae in the Netherlands the following question was asked: Which process to produce biodiesel from microalgae will be most suitable in the Netherlands, looking at strain selection, process technology and sustainability? This thesis focuses only on photoautotrophic microalgae grown in closed photo-bioreactors (PBRs) for the production of biodiesel in the Netherlands.

Multi criteria analysis (MCA) was used to determine the most suitable species by using the criteria: lipid productivity, nutrient usage, climatic suitability, content of valuable co-products and harvesting ease. Also for the process design (cultivation method & harvesting technique) an MCA was used with the criteria: land-use, construction costs, operational costs and efficiency. It turned out that *Nannochloropsis gaditana* cultivated in a vertical column PBR and harvested through filtration is the best option, although for larger microalgae species centrifugation is preferred.

The sustainability (minimizing environmental impacts and decreasing the depletion rate of fossil fuels) of the algae to biodiesel life-cycle was reviewed through a literature study focusing on: net energy rate (NER), greenhouse gas (GHG) balance, freshwater consumption, nutrient usage and co-product allocation. Microalgae cultivation inside a PBR, based on current PBR designs, is not sustainable due to elaborate PBR construction materials, PBR operation and high nutrient requirements. However, through process integration, biodiesel from microalgae can become a sustainable biofuel. Sea or wastewater should be used to provide nutrients while flue gas from power plants should provide CO<sub>2</sub>. The residual biomass should be used to generate electricity through anaerobic digestion, afterwards all the nutrients, and water, should be recycled. In the Netherlands a PBR would require additional heating, which should preferably be derived as waste heat from other industries. All these process integrations require more research before successful application.

Although the cultivation of microalgae has far higher productivities compared to other fuel crops, the cultivation (in PBRs) still requires large surface areas to give a substantial contribution to the Dutch policy goal of a 14% share renewable energy in 2020. This is however even a larger problem with 1<sup>st</sup> and 2<sup>nd</sup> generation biofuels indicating that microalgal biodiesel has a larger potential to become the biofuel from the future. The costs of 1 l microalgal biodiesel grown in a PBR are currently too high.

The Netherlands cannot become a large producer of microalgal biodiesel, mainly due to limitations in space. With higher productivities (l/ha/yr) some significant amounts for national consumption could possibly be commercial produced in a near future, although much research is needed especially within the field of genetic engineering and for the integration of sea/waste water and flue gas. The Netherlands could become a major consumer of microalgal biodiesel produced in other countries which have sufficient space and a better suitable climate. The Netherlands also do have the potential to become a source of knowledge for algae cultivation. To gain knowledge about algae cultivation the first PBRs should be focused on the production of valuable chemicals.

## Introduction

### *Problem outline*

A growing world population together with the development of new emerging economies like China and India will probably lead to a 54% increase in primary energy consumption by 2030 compared to 2007. This energy need should not and cannot be satisfied with only fossil fuels due to shrinking reserves and increasing environmental impacts. [IEA, 2009; FAO, 2010] In theory renewable energy sources provide a solution although much research is needed before realization. Microalgae have been proposed as a promising source for biofuels production [Scott et al., 2010]. This thesis focuses on the future of biodiesel production from microalgae in the Netherlands. The main question addressed is: Which process to produce biodiesel from microalgae will be most suitable in the Netherlands, looking at strain selection, process technology and sustainability?

### *Framing*

As space is very limited in the Netherlands focus will be only on closed photo-bioreactors (PBRs) because open ponds require, due to lower productivities, larger surface areas to meet the demands [Lehr & Posten, 2009]. This thesis is limited to microalgae (and cyanobacteria) which can be cultivated photoautotrophic, thus excluding macroalgae (seaweeds) and heterotrophic cultivation. The sustainability focuses only on minimizing environmental impacts and decreasing the depletion rate of fossil fuels but not on social aspects. Furthermore only biodiesel production is taken into account and not the production of other fuels. Also no economic analyses will be performed, although the costs of different production processes and operational costs will be considered.

### *Current energy use and CO<sub>2</sub> emissions*

The total primary energy supply (TPES) is the total amount of energy used annually (production + imports – exports) by a particular country or region. The total final consumption (TFC) is the amount of energy which is available for consumption. The main reason

**table 1:** Energy use in the World and in the Netherlands. Total primary energy supply (TPES), total final consumption (TFC) and associated CO<sub>2</sub> emissions in 2009 [IEA, 2011; EC, 2011; EL&I, 2011].

	TPES (TJ)	TFC (TJ)	CO <sub>2</sub> emissions (Million tonnes)
World	$5.09 \cdot 10^8$	$3.50 \cdot 10^8$	28999
NL	$3.35 \cdot 10^6$	$2.09 \cdot 10^6$	176

TPES is higher is because the energy required to produce energy is included in TPES (**table 1**) [IEA, 2011]. Worldwide 81.78% of the TPES was derived from fossil fuels (coal, oil and natural gas) leading to emissions of 28999 Mt of CO<sub>2</sub> in 2009. Especially the use of coal, peat and oil contributed to these emissions. In the Netherlands the TPES was estimated  $3.35 \cdot 10^6$  TJ with associated CO<sub>2</sub> emissions of 176 Mt (**table 1**) [IEA, 2011; EL&I, 2011]. Total worldwide CO<sub>2</sub> emissions are much higher as deforestation and other processes are not included. Fossil fuel prices have increased from \$20/barrel of crude oil (159 l) in the 1990s to a current (May 2012) level of \$90/barrel [Oil-price.net, 2012]. This is mainly caused by reductions in crude oil reserves and political conflicts. This together with the problems of global climate change (partially) caused by increasing greenhouse gas emissions (GHGs) have resulted in an increased interest in (new) renewable forms of energy.



### *Policies*

The European Union (EU) has set targets for 2020 to become more sustainable [EU, 2009 ;EL&I, 2011]:

- Increase the share of renewable energy sources in the total primary energy supply to 20%.
- Reduction of the greenhouse gas emissions by 20% compared to 1990.
- 20% increase in energy efficiency compared to 2000.
- CO<sub>2</sub>-poor economy in 2050

To meet these targets the EU set different targets for each of its member states, for the Netherlands a target of 14% of the TPES to be derived from sustainable energy sources is set for 2020. A CO<sub>2</sub>-poor economy in 2050 means a reduction in CO<sub>2</sub> emissions of 80-95% compared to 1990. To meet these targets the Dutch government wants to spend from 2015, €1.4 billion annually to stimulate the production of sustainable energy. Furthermore they want to oblige the co-firing of biomass in power plants. The Dutch government considers nuclear energy also as an option to reach their targets. [EL&I, 2011]

### *Renewable energy sources used today*

Some forms of renewable energy sources being used today are: solar-, wind-, hydro-, hydrogen- and geothermal-energy and energy from biomass. In 2009, worldwide, these renewable energy sources contributed 13.33% to the TPES [IEA, 2011]. Biomass is the largest contributor and can consist for example of agricultural and forest residues, animal manure, energy crops, organic wastes and algae. Especially in rural areas in developing countries biomass (wood) is important as it is often the only energy source available for cooking and heating [Demirbas & Demirbas, 2010]. In the Netherlands renewable energy sources contributed only 3.8% (equal to  $8.6 \cdot 10^4$  TJ) to the TPES in 2010 [CBS, 2010; EL&I, 2011]. Renewable energy sources consisted for the largest part of biomass, which accounted for 74.4% followed by wind energy (18.8%), geothermal energy (2.8%), outside air use (2.2%), solar (1.4%) and hydro energy (0.5%). This energy was primarily used in the form of electricity and heat and in a lesser extend for transportation. In the Netherlands biomass is used to produce energy by different processes, the most important are respectively: co-firing in power plants, wood stoves in households, waste incinerators and biofuels for transportation. [CBS, 2010]

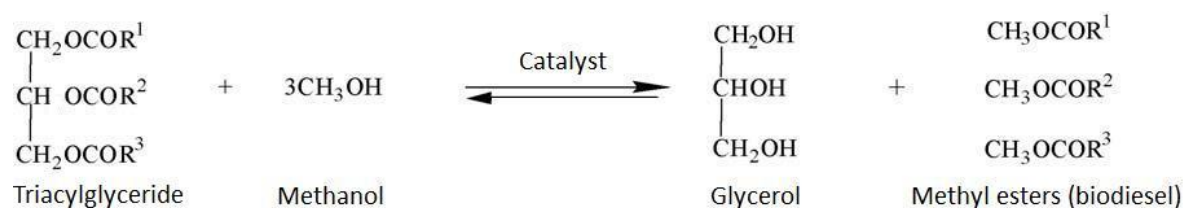
### *Biofuels*

Biofuels are; "solid, liquid, or gaseous fuels that are predominantly produced from biorenewable or combustible renewable feedstocks" [Demirbas & Demirbas, 2010]. The most commonly used biofuel is bio-ethanol which is produced through fermentation of plant derived sugars. This can be done directly or after the breakdown of cellulose. This fuel is a substitute for gasoline or can be mixed with gasoline. In 2010 the U.S. produced nearly 50 billion liters of corn ethanol. [Biello, 2011] Another important form of biofuel is biogas (main component is methane (CH<sub>4</sub>)) which is produced through anaerobic digestion of biomass by bacteria. This fuel can be used in gasoline vehicles with slight adaptations. [Naik et al., 2010] The third important biofuel is biodiesel, this is the main focus of this thesis as algae mainly produce this form of biofuel. There are different forms of biofuels available like hydrogen, bio-oil and Fischer-Tropsch fuels with their own production processes as well as different production pathways for the listed fuels, for a more in depth description see Naik et al (2010). [Naik et al., 2010]

### *Biodiesel*

Biodiesel is defined as "the monoalkyl esters of vegetable oils or animal fats" [Demirbas & Demirbas, 2010]. These monoalkyl esters are traditionally produced from triglycerides (also known as

triacylglycerides or TAGs) through transesterification. Triglycerides are large molecules that plants, for example soybean and jatropha but also algae use for storing (excess) energy. These molecules are located within the cells and need to be extracted. Transesterification is the process of exchanging the organic rest group (R) of an ester with the organic rest group of an alcohol often with the use of a catalyst. The main alcohol being used for production of biodiesel is methanol (CH<sub>3</sub>OH) while commonly used catalysts are sodium- and potassium- hydroxide (NaOH and KOH), all due to their low costs [Chisti, 2007]. The entire reaction is shown in **Equation 1**. Biodiesel is often mixed with petroleum diesel and used in normal diesel engines but can also be used pure in slightly adapted diesel engines [Naik et al., 2010].



**Equation 1:** *Transesterification reaction to produce biodiesel.* Triglycerides are converted into biodiesel and glycerol by using large amounts of methanol and a catalyst, adapted from Naik et al., (2010).

### *1<sup>st</sup> and 2<sup>nd</sup> generations biofuels*

Biofuels can be categorized as 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> generation biofuels. The 1<sup>st</sup> generation biofuels are made from food crops with high sugar and/or starch content, animal fats and vegetable oils. This category includes for example corn ethanol and soybean biodiesel. These biofuels directly compete with food and animal feed leading to increased food prices. The cultivation requires large areas of arable land, large input of fertilizers, pesticides and fresh water. [Miller, 2010] For example the large scale (49.2 billion litres annually) production of corn ethanol in the U.S. requires 40% of all corn fields and has led to an increase in food prices [Biello, 2011]. Furthermore soybean diesel and palm oil production are major contributors to deforestation in the Amazon and parts of Asia leading to additional CO<sub>2</sub> emissions and loss of biodiversity [Barona et al., 2011; Carlson et al., 2012]. 2<sup>nd</sup> generation biofuels are made from (herbaceous) non-food crops like jatropha, switch grass, willow and poplar. The biofuels in this category are mostly made from cellulose. These biofuels do not directly compete with food although they still compete for arable land. Some crops e.g. jatropha can grow on land unsuitable for food production. With these biofuels there are also still problems with fertilizers, pesticides and freshwater input but they do have a higher net energy balance and lead to higher GHG reductions [IEA, 2004]. Other problems are that cellulose is difficult and expensive to breakdown and that the techniques are still in their research stage. [Junginger, 2010; Biello, 2011]

### *3<sup>rd</sup> generation biofuels*

The 3<sup>rd</sup> generation biofuels consists only of algae based biofuels. This can be in the form of methane produced by anaerobic digestion of the biomass or photobiological produced biohydrogen, but mainly in the form of biodiesel derived from lipids in the algae. The use of algae for biodiesel production has already been researched since 1955 [Meier, 1955], but did get increased attention lately as fuel prices rose to record levels. Theoretical these biofuels have great potential because algae have higher growth rates compared to terrestrial plants and large amounts of biomass could be produced on relatively small areas of land [Chisti, 2007]. These advantages lead to higher annual oil yields per hectare compared to 1<sup>st</sup> and 2<sup>nd</sup> generation energy crops (**table 2**). Furthermore these algae can be grown on

marginal lands and in saline or brackish waters where there is not much competition with other uses. Algae can use nutrients from wastewater (providing bio-remediation) and use CO<sub>2</sub> from emitted flue gas from fossil fuel-fired power plants and other sources leading to decreased GHG emissions. [Hu et al., 2008]

### *Research questions*

The main question addressed is: Which process to produce biodiesel from microalgae will be most suitable in the Netherlands, looking at strain selection, process technology and sustainability? This question has been subdivided into multiple research questions.

- Chapter 1 (strain selection): Which biological characteristics of microalgae are important for producing biodiesel?
- Chapter 2 (process technology): What production process will technological be most suitable in the Netherlands?
- Chapter 3: What are the sustainable opportunities and risks of this process?
- Chapter 4: What are the major obstacles in the realization of commercial biodiesel production and what possible solutions are available?

### *Approach & Methodology*

To determine if and how biodiesel production from microalgae will be possible in the Netherlands in the future, four major aspects of the process will be discussed: strain selection, process technology, sustainability and challenges in scaling-up. Where sustainability in this thesis refers to the sustainability on the environment by both minimizing impacts and by decreasing the rate of fossil fuels use. Social sustainability (e.g. human rights and labour rights) is not taken into account. Each of these four aspects will be dealt with in a separate chapter. In chapter 1 & 2 the most suitable option for biodiesel production in the Netherlands will be determined by making use of multi criteria analyses (MCAs). The criteria being used in these MCAs are for chapter 1 (strain selection): lipid productivity, nutrient usage, climatic suitability, content of valuable co-products and harvesting ease. For chapter 2 (process technology): land use, construction costs, operational costs and efficiency of the design are used. All criteria are assessed on a scale from ++ to --, where ++ is most favourable and -- least favourable. In the third chapter (sustainability) the sustainability of biodiesel production from microalgae is discussed with the focus on the NER, GHG balance, freshwater consumption, nutrient usage and co-product allocation. In chapter 4, challenges in scaling-up are discussed looking at the major problems that prevent the realization of commercial biodiesel production from algae. This thesis ends with a discussion and conclusion about the future of biodiesel production from microalgae in the Netherlands. In this discussion a SWOT analysis has been made to compare microalgal biodiesel to 1<sup>st</sup> and 2<sup>nd</sup> generation biofuels. Based on the outcomes of this SWOT analysis an optimistic view and sceptical view on the future of biodiesel production from microalgae in the Netherlands are given.

**table 2; Estimations of oil yields.** A comparison of oil yields in l/ha/yr from different energy crops being currently used for biodiesel production, based on; Chisti, 2007; Waltz, 2009; Scott et al., 2010; Mata et al., 2010.

<b>Fuel crop</b>	<b>Oil yield (l/ha/yr)</b>
Corn	172
Soy	402-636
Sunflower	804-1070
Canola	974-1599
Jatropha	741-2700
Oil palm	5366-5993
Microalgae	8200≤

## 1. Stain selection

### Introduction

An important aspect of the biodiesel production process is the choice of algae species. Algae are a large and highly diverse group which can be found on all earths ecosystems. Microalgae are unicellular algae which are used for biodiesel production. Macroalgae, seaweeds, are also being cultivated but mainly as fertilizers or for their polysaccharides which are used as gelling or thickening agents. Their cultivation takes place off-shore [Pulz & Gross, 2004]. There are an estimated 50.000 species of microalgae, from which more than 30.000 species have been already indentified [Richmond, 2004]. Especially in the U.S. aquatic species program from 1978 till 1996 large efforts have been made towards finding suitable microalgae species for biodiesel production. 3000 strains were collected from which 300 species (mostly green algae and diatoms) were further investigated. [Sheehan et al., 1998] To date the perfect microalgae for biodiesel production still has not been found. The question addressed in this chapter is: Which microalgae, with which biological characteristics, are most suitable for biodiesel production? The suitability of different species of microalgae is assessed according to five criteria: lipid productivity, nutrient usage, climatic suitability, content of valuable co-products, ease of harvesting. These criteria are chosen as I believe these microalgal characteristics contribute most to the sustainability and economic viability of the production process. The criteria are assessed on a scale from - - (least suitable) to + + (most suitable).

### Guilds

Strictly microalgae are eukaryotic organisms but in this thesis (like in most literature) prokaryotic cyanobacteria (blue-green algae) are also included. Microalgae being used in literature concerning biodiesel production can be classified in multiple major groups: cyanobacteria (Cyanophyceae), green algae (Chlorophyceae), red algae (Rhodophyceae), diatoms (Bacillariophyceae), brown algae (Phaeophyceae) and pico-plankton (Eustigmatophyceae) (**Figure 1**). [Hu et al., 2008; Scott et al., 2010; Radakovits et al., 2012] Most microalgae are photoautotrophic organisms which use sunlight as energy source to turn inorganic carbon (CO<sub>2</sub>) into chemical energy. Some microalgae can also use different forms of metabolism e.g. heterotrophic, using organic matter as energy and carbon source or mixotrophic where sunlight is used as energy source but both organic and inorganic carbon are being used as carbon source [Chen et al., 2011].



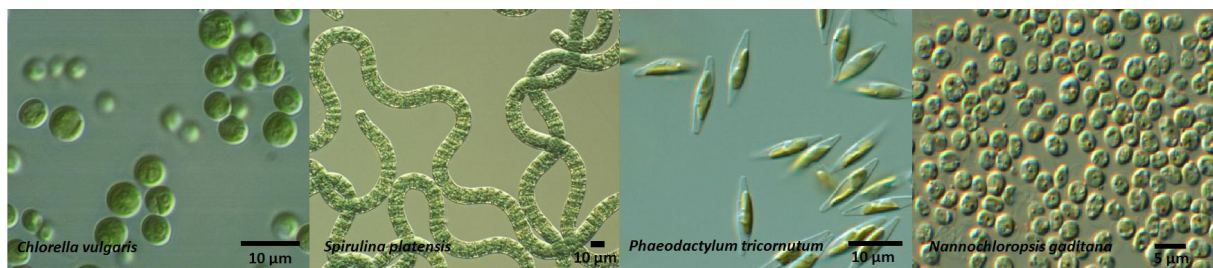
**Figure 1;** *Microalgae species in this thesis.* Simplified schematic phylogenetic tree of photosynthetic microalgae with species of interest for biodiesel production. Based on Hu et al., 2008; Scott et al., 2010; Radakovits et al., 2012.

Research has shown that species grown in heterotrophic cultivation can produce up to 20 times more lipids than species grown in autotrophic cultivation [Chen et al., 2011]. *Chlorella protothecoides* grown

under heterotrophic conditions showed a 4 times higher lipid content compared to when grown under autotrophic conditions [Xu et al., 2006]. Also mixotrophic cultivation leads often to higher growth rates [Brennan & Owede, 2010]. These cultivation methods require additional organic carbon input making these methods less interesting for this thesis as resource use is higher and also less (atmospheric) CO<sub>2</sub> can be mitigated.

### Species of interest

For this thesis a small selection of microalgae species was made to be used in the MCA: The freshwater green algae *B. braunii*, *C. vulgaris* and *H. pluvialis*, the marine green algae *D. salina*, the marine pico-plankton *N. gaditana*, the marine diatom *P. tricornutum* and the cyanobacterium *S. platensis*. These species were chosen because their prominent place in literature. This is either because the species are already being used for the production of valuable chemicals or because their high potential for biodiesel production. These species are relatively well known. [Rodolfi et al., 2009; Mata et al., 2010; Chen et al., 2011; Larkum et al., 2011] **Figure 2** shows images of some of the microalgae being used (one species of each major group).



**Figure 2;** Images of microalgae. From left to right; the green algae *C. vulgaris*, the cyanobacteria *S. platensis*, the diatom *P. tricornutum* and the pico plankton *N. gaditana*. Scale is 10 µm except for the 4<sup>th</sup> image which is 5 µm. Image 1 and 2 [SHIGEN, 2012], image 3 and 4 [NCMA, 2012].

### Lipid productivity

In general, it appears that algae produce triglycerides at times when the energy input, through carbon assimilation, exceeds the immediate metabolic needs of the cell [Greenwell et al., 2009]. Additional amounts of triglycerides are synthesized when growth is limited by external stress factors like nutrient limitation [Stephenson et al., 2010b]. These triglycerides are then stored until favourable conditions

**table 3;** Lipid content, biomass productivities and lipid productivities of different photoautotrophic microalgae. Scores are given based on the relative differences between the species. Based on Chisti, 2007; Rodolfi et al., 2009; Mata et al., 2010; Xue et al., 2010; Chen et al., 2011; Brennan & Owede, 2010; Gouveia & Oliveira, 2009, Radakovits et al., 2012.

Species	Lipid content % of dry weight	Biomass productivity (g/l/day)	Lipid productivity (mg/l/day)	Score
<i>Botryococcus braunii</i>	14 - 75	0.02 - 0.077	5.5	- -
<i>Chlorella vulgaris</i>	5 - 58	0.02 - 0.20	40	0
<i>Dunaliella salina</i>	6 - 25	0.22 - 0.34	116	+
<i>Haematococcus pluvialis</i>	25	0.05 - 0.41	15	-
<i>Nannochloropsis gaditana</i>	47	0.65	310	++
<i>Phaeodactylum tricornutum</i>	19 - 30	0.24	44.8	0
<i>Spirulina platensis</i>	4 - 16	0.06 - 0.42	24	-

return to be quickly mobilized again [Greenwell et al., 2009]. This means that increasing lipid content will not necessarily result in increased lipid productivity because lipid accumulation takes place at the expense of biomass productivity [Sheehan et al., 1998]. In **table 3** the lipid content, biomass productivity and the lipid productivity are shown. Most important is the lipid productivity, as this gives insights in the efficiency for biodiesel production.

#### *Nutrient usage*

All photoautotrophic microalgae require, next to sunlight, Nitrogen (N), Phosphorus (P), CO<sub>2</sub> and some trace metals (e.g. Fe, Mg and Ca) to grow. Diatoms require additional silica for growth. The production of nutrients is a highly energy-consuming process making nutrient usage an important aspect considering the sustainability of the entire production process [Yang et al., 2011]. Next to maximum nutrient recycling, it is often proposed to combine the production with sea or wastewater (for nutrients) and flue gas (for CO<sub>2</sub>) treatment to minimize the need for special produced nutrients [Vunjak-Novakovic et al., 2005; Mata et al., 2010]. Ideally algae grown in wastewater and by using flue gasses would not require any other nutrient inputs. Algae have been shown to successfully treat livestock wastewaters, large amounts of N, P and other metals were removed and algae showed increased growth rates [Kebede-Westhead et al., 2006]. Flue gas has high concentrations of CO<sub>2</sub>, up to 30%, which in some species can lead to increased growth [McGinn et al., 2011]. Not all species are suitable for CO<sub>2</sub> mitigation as CO<sub>2</sub> levels might be too high and flue gasses can also contain toxic components like NO<sub>x</sub> and SO<sub>x</sub> [Yoo et al., 2010].

*B. braunii* can efficiently use nutrients from wastewater [Shen et al., 2008] and has been shown to have high lipid productivities when grown with flue gas as CO<sub>2</sub> source [Yoo et al., 2010]. *C. vulgaris* has also been shown to successfully sequester carbon from flue gas [Keffer & Kleinheinz, 2002] and grow in wastewater [Kim et al., 2010]. *D. salina* can only be used to treat wastewater if immobilized [Mallick, 2002]. Furthermore this species is highly productive when flue gas is used as CO<sub>2</sub> source [Zimmermann et al., 2011]. *H. pluvialis* has a lower score as it is only able to grow on diluted wastewater [Kang et al., 2006] and cannot tolerate high CO<sub>2</sub> levels [Meiser et al., 2004]. Research has not shown the potential of *N. gaditana* for wastewater or flue gas treatment. *S. platensis* is able to grow in wastewater but is not efficient in nutrient removal [Mezzomo et al., 2010] *S. platensis* is also not used for flue gas treatment. *P. tricornutum* growth was inhibited with CO<sub>2</sub> levels above 2% [Meiser et al., 2004]. This species is however capable of wastewater treatment and normally grows in seawater [Goldman & Stanley, 1974]. Scores are shown in **table 5**.

#### *Climatic suitability*

Microalgae live in different environments with different climatic parameters. Algae differ in their optimum light intensity and temperature for maximum growth. In the Netherlands the light intensity and the average temperature are relatively low. An algae which is able to achieve high productivities in this climate would be preferred to minimize the burdens of additional illumination and heating. An microalgae can easily tolerate temperatures of up to 15 °C below their optimum but more than 5 °C above their optimum can be fatal [Mata et al., 2010]. Microalgae are much more efficient in utilizing incoming sunlight compared to terrestrial plants. The theoretical maximum conversion factor from the photoactive radiation (PAR) is 26.7%, which means that 26.7% of the PAR can be converted into biomass. [Weyer et al., 2010] Some microalgae have been shown to have a conversion factor of almost 10%, compared to 1-2% from the “highly productive” energy crop *Miscanthus* [Heaton et al., 2008;



Brennan & Owede, 2010]. Light intensities which are too high lead to cell damage [Weyer et al., 2010]. However this problem will be relatively small in the Netherlands. In the table below the scores for climatic suitability are given (**table 4**).

**table 4:** *Climatic suitability.* Optimal light intensity and optimal temperature (range) are combined to one score for climatic suitability. Low light intensity is <100, medium 100-200 and high 300<  $\mu\text{mol photons/m}^2/\text{s}$ . Scores from ++ to - -.

Species	Optimal light intensity	Optimal temperature (range)	Score	Source
<i>B. braunii</i>	Low	23 °C	+	Qin & Li, 2006
<i>C. vulgaris</i>	Medium	25-30 °C	-	Dauta et al., 1990
<i>D. salina</i>	High	33 °C	- -	Borowitzka et al., 1984
<i>H. pluvialis</i>	Low	25-28 °C	+	Fan et al., 1994
<i>N. gaditana</i>	Medium	20-30 °C	0	Rocha et al., 2003
<i>P. tricornutum</i>	Low	21-25 °C	+	Fawley, 1984
<i>S. platensis</i>	High	30-37 °C	- -	Kebede & Ahlgren, 1996

#### *Content of valuable co-products*

As economic viability is still the biggest problem in the realization of commercial biodiesel production from algae. The production of valuable co-products can contribute to a more economic viable production process. Many of the species from the selection have already been used to produce products for human consumption. *B. braunii* can be used to produce hydrocarbons and triterpene oils [Metzger & Largeau, 2005]. *D. salina* is used at large scale to produce the valuable  $\beta$ -carotene [Smith et al., 2010]. *P. tricornutum* produces omega-3 fatty acids and eicosapentaenoic acid (EPA) which are good for human development [Yongmanitchai & Ward, 1992]. *N. gaditana* can also produce EPA but the production of this polyunsaturated fatty acid goes at the expense of lipid production. [Spolaore et al., 2006; Brennan & Owede, 2010]. *H. pluvialis* is currently being used to produce the highly valuable astaxanthin, which is a carotenoid being good at protecting lipids against peroxidation and has other health benefits. [Brennan & Owede, 2010] Cyanobacteria are known to produce at least 50 compounds which are toxic to vertebrates [Carmicheal, 2008]. However, no research has shown that *S. platensis* contains toxic components. Dried *Spirulina*, as a whole, has for many years been used as healthy food, feed and medicine. It does not however produce specific valuable compounds [Li & Qi, 1997]. *C. vulgaris* does not contain valuable co-products. Scores are given in **table 5**.

**table 5:** *Overview of all scores used for MCA.* Scores for the 5 chosen criteria, scores range from - - to ++.

Species	Lipid productivity	Nutrient usage	Climatic suitability	Harvesting ease	Valuable co-products
<i>B. braunii</i>	- -	++	+	+	+
<i>C. vulgaris</i>	0	++	-	0	0
<i>D. salina</i>	+	0	- -	0	++
<i>H. pluvialis</i>	-	- -	+	-	++
<i>N. gaditana</i>	++	-	0	- -	+
<i>P. tricornutum</i>	0	0	+	-	+
<i>S. platensis</i>	-	0	- -	++	0

### Ease of harvesting

The harvesting of the microalgae followed by the separation of cell compartments is a major cost in the production process. The harvesting method depends on the size of the microalgae, larger species will be easier (and cheaper) to harvest. [Mata et al., 2010] Some species will form colonies which are easier to harvesting, species which naturally do not form colonies can be stimulated to flocculate. Some species have thicker cell walls making the separation of the different products (oil) harder. *S. platensis* is the largest ( $\pm 70 \mu\text{m}$ ) microalgae in this selection making it most suitable for harvesting. *B. braunii* is relatively small ( $< 30 \mu\text{m}$ ) but forms colonies by nature. *D. salina* (0) and *C. vulgaris* are also both small ( $< 30 \mu\text{m}$ ). *P. tricornutum* has a similar size ( $< 30 \mu\text{m}$ ) but has a hard cell wall making cell disruption more difficult. *N. gaditana* is the smallest species (2-5  $\mu\text{m}$ ) and also has a hard cell wall making it the most expensive species to harvest [Bailliez et al., 1985; Rodolfi et al., 2009]. *H. pluvialis* has a thick cell wall as well [Suh et al., 2006]. [Brennan & Owede, 2010] Scores for ease of harvesting are given in **table 5**.

**table 6:** Multi criteria analysis for best suitable microalgae for biodiesel production. For each criterion the ++ to -- scores are converted to scores between 0 and 1 and multiplied by their given weight (shown after the criteria). Lipid productivity is the highest weighted criterion to be multiplied by 3 while content of valuable co-products is weighted as lowest (0.5). *N. gaditana* turns out to be most suitable microalgae from this selection according to this MCA.

Species	Lipid productivity (3)	Nutrient usage (2)	Climatic suitability (2)	Harvesting ease (1)	Valuable co-products (0.5)	Total
<i>B. braunii</i>	0	2	1.5	0.75	0.375	4.625
<i>C. vulgaris</i>	1.5	2	0.5	0.5	0.25	4.75
<i>D. salina</i>	2.25	1	0	0.5	0.5	4.25
<i>H. pluvialis</i>	0.75	0	1.5	0.25	0.5	3
<i>N. gaditana</i>	3	0.5	1	0	0.375	<b>4.875</b>
<i>P. tricornutum</i>	1.5	1	1.5	0.25	0.375	4.625

### Multi criteria analysis

An MCA was carried out to determine which microalgae species scores best when all five aspects are considered. The five different criteria are weighted according to their importance for biodiesel production. Lipid productivity (3) is considered most important as this is directly influencing biodiesel production. High productivity is needed to minimize the surface area required. Nutrient usage (2) and climatic suitability (2) are weighted second most important as these two factors determine to a great extent the costs and sustainability of the process. Harvesting ease (1) is valued less important as it does contribute to the sustainability of the process but many other techniques are already known which might make this criterion redundant. The production of valuable co-products (0.5) is valued to be the least important as the production of these co-products will often go at the expense of biodiesel production, require different, additional, extraction methods and do not contribute to the sustainability of the process. From this MCA it turns out that *Nannochloropsis gaditana* is the best suitable species for biodiesel production in the Netherlands (**table 6**).



### *Sensitivity analysis*

The outcome of this MCA is dependent on the values chosen for weighting the criteria. When the criteria are not weighted *B. braunii* would have had the highest overall score followed by *C. vulgaris*, *D. salina* and *P. tricornutum*. Also with other weights *B. braunii* often has the highest score (often followed by *C. vulgaris*). *B. braunii* would be ideal for cultivation in the Netherlands but cannot be used for biodiesel production with these low lipid productivities. However the large amount of hydrocarbons produced can also be used as fuel (gasoline or jetfuel) [Sakamoto et al., 2012] This is however outside the scope of this thesis. The two species which in different weighting scenarios score good, *N. gaditana* and *C. vulgaris*, are both promising candidates.

### *Ideal species*

According to Brennan & Owede (2010) the ideal microalgae, which at this moment does not exist, will also be resistant to shear stress, tolerant to wide range of temperatures resulting from the diurnal cycle and seasonal variations and have a high light efficiency [Brennan & Owede, 2010]. These factors are not taken into account as I believe biodiesel production in the Netherlands has to be indoors (greenhouses) in a controlled environment due to a highly variable climate, making these traits superfluous. As currently not a single species lives up to our expectations genetic engineering could be the solution. To date only a few species have been genetically modified, from which *Nannochloropsis* sp. and *Dunaliella* sp. are two promising species [Larkum et al., 2011]. For one species in particular, the green algae *Chlamydomonas reinhardtii* (which does not contain lipids suitable for biodiesel production), many techniques are available for genetic modification. For example, Melis (2009) has succeeded in increasing the photosynthetic efficiency by modifying the chlorophyll antennae [Melis, 2009]. By learning from this species efforts can be made to modify the characteristics limiting commercial biodiesel production. According to Wijffels & Barbosa (2010) the ideal microalgae will have large cells with thin membranes, grow fast under high light conditions while producing maximum amounts of lipids, are resistant to high levels of O<sub>2</sub>, are robust and resistant to infections, form flocs and, maybe most important, excrete their oils [Wijffels and Barbosa, 2010]. To realize this microalgae much research will be needed.

## 2. Technological Production Process

### *Introduction*

The production of biodiesel can take place in two major systems; open ponds or closed photo-bioreactors (PBRs). Open ponds are often used to cultivate microalgae because of their low construction and operational costs. To overcome their most negative aspect, contamination or predation by other unwanted organisms, algae are often grown in highly species specific conditions like high saline water for *D. salina* or high alkaline water for *S. platensis* [Chisti, 2006]. Another problem is often water loss by evaporation. Stephenson et al., (2010a) however have found that water input by rainfall will be higher than the loss due to evaporation in the UK, which has a similar climate as the Netherlands [Stephenson et al., 2010a]. Open ponds are always shallow because light can only penetrate to 30 cm into the water. Therefore large scale production of biodiesel requires a huge surface, which is an issue in the Netherlands. Also by using open ponds no genetically engineered organisms could be used. In this thesis only PBRs are considered as possible options for biodiesel production in the Netherlands. The question in this chapter is: What production process will be most suitable in the Netherlands? The suitability of the production process will be determined based on PBR design and harvesting method. Different options for these two aspects are assessed according to four criteria: land-use, operational costs, construction costs and efficiency. These criteria are chosen because together they determine the suitability in the Netherlands but also, in some extent, the sustainability and the economic viability. The criteria are assessed on a scale from - - (least suitable) to + + (most suitable).

### *Production process*

The production process can be divided into four major stages: cultivation, harvesting, extraction and conversion [Mata et al., 2010]. First large amounts of biomass need to be grown under optimal conditions while nutrients need to be supplied. Then the biomass needs to be harvested from the medium. The cells in the recovered algae slurry need to be disrupted (unless lipids could be secreted) to extract the lipids. These are then converted to biodiesel through transesterification while the residual biomass will be processed further or recycled.



**Figure 3:** Different photo-bioreactor (PBR) designs. A: Tubular PBR [BRAE, 2012], B: Flat plate PBR [Bitog et al., 2011], C: Vertical-column PBR [Silipa, 2012].

### *Closed photo-bioreactor designs*

In this thesis three of the most common, basic, PBR designs are taken into account: flat plate, tubular and vertical-column PBRs (**Figure 3**). An important aspect of each PBR is mixing, this is needed to prevent sedimentation and algae attachment to the walls of the PBR. But also to allow equal light

intensity for all algae, keep equal levels of CO<sub>2</sub> and to avoid photo-oxidation. Another important design aspect is light penetration as the diameter, placement and other design aspects of a PBR are all dependent of this. [Bitog et al., 2011] Because PBRs are closed systems there is a problem with excess O<sub>2</sub> produced by respiration this causes the process of photo-oxidation which lead to cell damage. To prevent photo-oxidation PBRs need to have a degassing area to remove O<sub>2</sub> from the culture. This, together with a pH gradient (caused by a gradually CO<sub>2</sub> uptake), are the main factors limiting the length of a PBR. [Chisti, 2007]

#### *Flat plate PBRs*

Flat plate PBRs are efficient (++) in biomass production as they have a large illuminated surface area leading to high biomass productivity per litre [Dragone et al., 2010]. The algae culture in this PBRs is mixed by an airflow at the base of the plate [Hu et al., 1996]. The amounts of dissolved O<sub>2</sub> concentrations are relatively low and the problems with pH gradients are moderate. Furthermore both construction (+) and operational costs (+) are relatively low [Dragone et al., 2010]. A negative aspect of these kind of PBRs is that a large surface area is required which especially is a problem with scaling up (land-use: -). It is also difficult to control the temperature and photo-inhibition (damage to the light harvesting complex due high light intensities) could take place on days with high levels of solar radiation, which would be of lesser concern in the Netherlands. Furthermore there is the possibility of shear stress. [Ugwu et al., 2008]

#### *Tubular PBRs*

Tubular PBRs are also relatively cheap in construction (+) and have a large illuminated surface area but their efficiency (+) to produce biomass is slightly lower compared to flat plate PBRs partly due to photo-oxidation and pH gradients [Dragone et al., 2010]. Tubular PBRs often have a diameter of around 10 cm to maximize sunlight penetration [Demirbas & Demirbas, 2010]. These PBR require extensive pumping to prevent wall growth, sedimentation and photo-oxidation leading to higher operational costs (-). For preventing photo-oxidation they also need a (separate) degassing column. Pumping is considered as the major contributor to operational costs and in a lesser extent to construction costs [Stephenson et al., 2010a]. Also tubular PBRs require larger amounts of land compared to the vertical columns (land-use: -). [Ugwu et al., 2008]

#### *Vertical column PBRs*

Vertical columns have a smaller illuminated surface area, but similar productivities can be reached as with tubular PBRs (efficiency: +) . There are two major types of vertical columns; bubble columns and air-lift columns. In bubble columns mixing is performed by bubbles starting at the bottom of the entire column, in the latter a current is created by an air flow into a pipe in the middle of the reactor. Due this kind of mixing operational costs are low (++) and levels of photo-oxidation and other gradients are minimal but there may be the problem of shear stress. [Miron et al., 2002] These PBRs have a ± 20cm diameter and could reach heights of 4 m which lead to a low land-use (+). These tall structures require more expensive material, resulting in higher construction costs (-). These PBRs have the best potential for scaling-up [Bitog et al., 2011]. Another advantage of this design is that it can easily be internally illuminated by a light source (LED), which can lead to a larger diameter of the PBR with higher yields, but also to higher operational costs [Ugwu et al., 2008; Chen et al., 2011]. An overview of all the scores of the PBRs are shown in **table 7**.

### *Harvesting*

After the cultivation of the algae the biomass needs to be harvested. Harvesting is the process of recovering the algae from the medium to lower the water content (typical to a 5-15% dry solid content) [Brennan & Owede, 2010]. Harvesting can be done per batch (discontinuous) or continuous, for a large scale production continuous harvesting is preferred. The three most common harvesting methods are sedimentation, centrifugation and filtration. [Grima et al., 2003] As discussed in the previous chapter the method of choice is mainly dependent on the algae strain but also on the density of the algae and the (low) value of the desired product (biodiesel). Harvesting contributes 20-30% of the total costs [Grima et al., 2003]. To make extraction of the different products possible, the water content has to be further decreased e.g. hexane extraction requires a moisture content of 9% [Sheehan et al., 1998; Patil et al., 2008]. This can be done by sunlight, which is not a good option in the Netherlands, or by thermal drying (spray drying) which is expensive [Grima et al., 2003; Mata et al., 2010].

Flocculation is an often used step to increase particle size before harvesting to make it more efficient. As algae are naturally negatively charged to prevent aggregation, different kinds of techniques are available to lower or neutralize this surface charge. This is primarily done by adding flocculants which are mainly metal salts. Ideally flocculants should be inexpensive, non-toxic, effective in low concentrations and downstream processing should not be affected. [Grima et al., 2003]

### *Sedimentation*

Sedimentation is the process whereby algae will settle on the bottom of the medium due to gravity. This method is only possible with larger (>70 µm) microalgae like *Spirulina* sp. and is relatively slow (efficiency: - -). It is often used to recover algae in sewage water treatments, because of the low construction (++) and operational (++) costs [Nurdogan & Oswald, 1996]. The biomass recovered, per batch, still has a high water content (1.5% dry solid content). Which, depending on the extraction method, will need to be dried intensively afterwards [Murthy, 2011]. Furthermore sedimentation requires a large sedimentation tank (land-use: - -) [Shelef et al., 1984].

### *Centrifugation*

Centrifugation is a quick and efficient (++) continuous method for recovering algae. It is as well only suitable for larger microalgae, but flocculation can be a solution for medium sized algae. The efficiency of centrifugation is depended of the algae and the rotation speed, high-speed centrifugation makes it possible to recover a >95% of the algae from the medium [Heasman et al., 2000]. This high speed centrifugation is only possible with tough algae, but would lower the need for drying afterwards. Another good, and less energy consuming, centrifuge is the so called "self-cleaning disk stack", this method yields algae paste with a 12% solid content. The construction costs (- -) of this type of harvesting are high while the operational costs (-) are also substantial due to high energy requirements [Sander & Murthy, 2010]. [Grima et al., 2003] Centrifugation may become a more economic viable method on larger scales [Mackay & Salusbury, 1988].

### *Filtration*

Normal filtration operates under pressure or vacuum and is suitable for only large species of microalgae. The most reliable and effective filtration method is the "Netzsch chamber filter", which can recover an algae paste with 22-27% solid content. This is an efficient method to harvest algae but

it is relatively slow (efficiency: +). Furthermore this methods has lower energy requirements than the “self-cleaning disk stack” but operational costs (-) are still higher due to membrane replacement. The construction costs (0) of filters are relatively low compared to centrifugation. [Wang et al., 2008] The disadvantage of filtration is the low speed of the process. Other forms of filtration e.g. ultra-filtration, is one of the few possibilities for harvesting small microalgae. These techniques to recover the smallest microalgae have higher production and operational costs due to the special membranes. [Grima et al., 2003]. The land-use (+) is similar to that of centrifugation. An overview of the strengths and weaknesses of these 3 harvesting methods are shown in **table 7**.

**table 7:** Basic scores for MCA. Strengths and weaknesses of the different PBR designs and harvesting methods are shown, score range from ++ to - - (see text for explanation).

PBR Design	Land-use	Construction costs	Operational costs	Efficiency
Flat plate	-	+	0	++
Tubular	-	+	-	+
Vertical-column	+	-	++	+
<b>Harvesting</b>				
Sedimentation	- -	++	++	- -
Centrifugation	+	--	0	++
Filtration	+	0	-	+

### Extraction

After the algae have been harvested, dried and reached the desired water content the cells need to be disrupted. cells can be disrupted chemical, mechanical or a combination of both, with sustainability in mind, mechanical disruption is preferred to avoid further chemical contamination [Greenwell et al, 2009]. Some possible mechanical options are; homogenization, bead milling, ultrasounds, autoclaving or combinations of these [Mata et al., 2010; Brennan & Owede., 2010]. Two studies found that the use of ultrasounds is the most effective way to disrupt algae cells [Prabakaran & Ravindran, 2011; Lee et al., 2010]. Another study found that high-pressure homogenization was the most effective method for disruption, although they did not take energy consumption into account [Halim et al., 2012]. The most used (chemical) method of extraction is the use of hexane, this is an efficient process but requires low water content of the algae biomass [Sheehan et al., 1998]. Kita et al., (2010) successfully extracted hydrocarbons from a wet algae biomass at 85 °C [Kita et al., 2010]. Teixeira also succeeded to disrupt cells in a wet biomass by using a ionic solution at 120 °C [Teixeira, 2012]. These are promising results to eliminate the expensive drying process. To determine what the best option is for extraction, including drying, in the Netherlands is out of the scope of this thesis.

### Conversion

After the extraction the lipids are converted into biodiesel by transesterification. There are other conversion processes e.g. gasification, anaerobic digestion and pyrolysis, which respectively produce syngas, methane and bio-oil. These techniques can be used for rest biomass to make the process more economic viable. [de Boer et al., 2012] The transesterification of the lipids require large amounts of methanol and the addition of a catalyst. Both the methanol and catalyst should be recycled to lower the costs. This is a challenge for searching the appropriate catalyst [Greenwell et al., 2009].

### Multi criteria analysis

An MCA is performed to determine which PBR design and which harvesting method would be most suitable for biodiesel production in the Netherlands. The criteria land-use is chosen to be most important, and weighted as 3, as biodiesel production would always require substantial amounts of surface area which is a major limitation in the Netherlands. Operational costs and construction costs are both weighted as 2 as these two will impact both the sustainability and economic viability of the process. Efficiency in the PBR designs is weighted as 1 because the differences are small. Efficiency for the harvesting technique is weighted as 2 because here the speed of the harvesting could be limiting to the amount of biodiesel produced, especially on large scales. In **table 8** the outcomes of the MCA are given.

**table 8:** Multi criteria analysis for best suitable production process design. For each criterion the ++ to -- scores are converted to scores between 0 and 1 and multiplied by their given weight (shown after the criteria). Land-use is the highest weighted criterion to be multiplied by 3. A vertical column PBRs where algae are harvested by filtration or by centrifugation turns out to be most suitable option for biodiesel production in the Netherlands according to this MCA.

PBR Design	Land-use (3)	Operational costs (2)	Construction costs (2)	Efficiency (1)	Total
Flat plate	1.5	1	1.5	1	5
Tubular	1.5	0.5	1.5	0.75	4.25
Vertical-column	2.25	2	0.5	0.75	<b>5.5</b>
Harvesting	(3)	(2)	(2)	(2)	
Sedimentation	0	2	2	0	4
Centrifugation	2.25	1	0	2	<b>5.25</b>
Filtration	2.25	0.5	1	1.5	<b>5.25</b>

### Sensitivity analysis

The vertical column PBR scores in most cases (with different weightings) the highest followed by the flat plate design. Only if both construction costs and efficiency would be weighted higher the flat plate design would score higher. In the case of harvesting technique the scores of centrifugation and filtration are close to each other. Only at construction costs centrifugation has a lower score. As construction costs are a single investment (depending on the life-time of the machinery), operational costs could be weighted higher. In this case centrifugation would have a higher score.

### Most suitable production process in the Netherlands

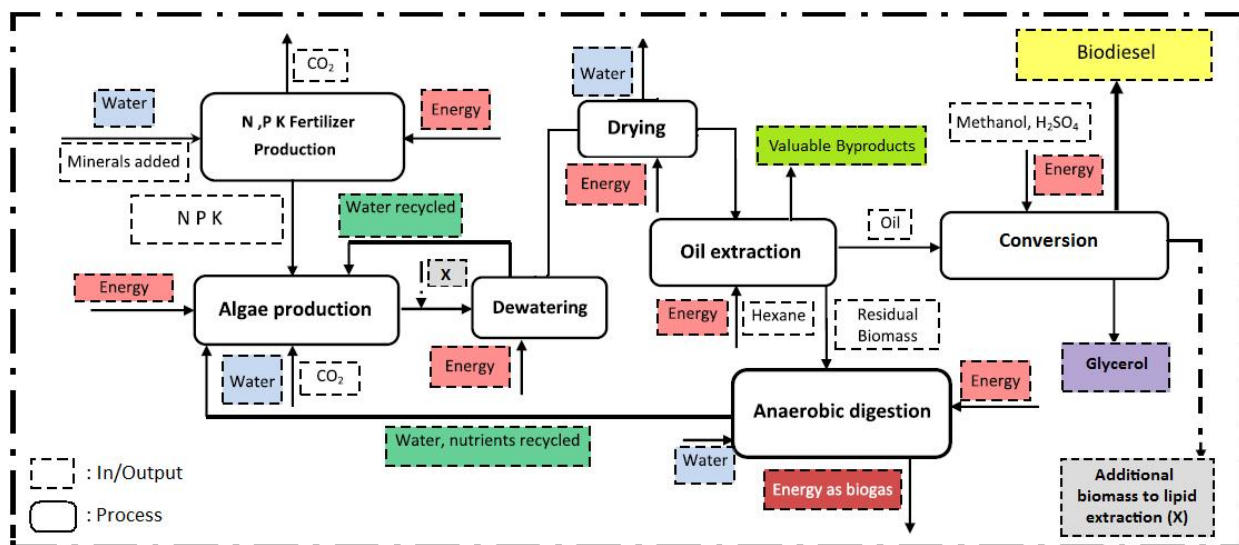
The MCAs indicate that the best PBR design in the Netherlands would be a vertical column PBR while the best harvesting technique would be filtration or centrifugation (**table 8**). The harvesting method should be adjusted to the species of microalgae used. As in the previous chapter *N. gaditana* turned out to be the most suitable species, filtration would be preferable due to its small cell size. However for larger species centrifugation would be preferred. The vertical column PBR has been proposed by multiple authors to have a great potential in the future [Ugwu et al., 2008; Bitog et al., 2011; Singh & Sharma, 2012]. The placement of the factory would preferably be near a power plant to allow usage of wastewaters, flue gasses and heat. Because of the highly variable weather conditions in the Netherlands combined with often low light intensities the bioreactors might need to be placed in a greenhouse with additional (internal) light sources and heating. This will add to the production and

operational costs. Also there are still many challenges left in making biodiesel production economic viable e.g. finding a cheap extraction and conversion method which does not require dried biomass. According to Singh & Sharma (2012) the production of biodiesel is currently not economic viable without additional subsidies [Singh & Sharma, 2012].

### 3. Sustainability

#### Introduction

The use of fossil fuels has led to increased atmospheric CO<sub>2</sub> concentrations which lead to other environmental problems like e.g. global warming, acidification of the oceans and the release of even more GHGs from soils [Knohl & Veldkamp, 2011; Witt et al., 2011]. The new generations of fuels should ideally be CO<sub>2</sub> neutral and have minimal impacts on the environment, in other words it should be sustainable. Where sustainability in this thesis refers to the sustainability on the environment by both minimizing impacts and by decreasing the rate of fossil fuels use. As in the previous chapter the technological aspects of the production process are discussed, this chapter will focus on the sustainability of the process. The question addressed is: What are the sustainable risks and opportunities of this process? Criteria analyzed are: net energy rate (NER), greenhouse gas (GHG) balance, freshwater consumption, nutrient usage and co-product allocation. These criteria were chosen because they are the major contributors to the sustainability of the biodiesel production process [Soratana & Landis, 2011; Grierson & Strezov, 2012]. Another important aspect would be the release of toxic components, this criterion however is not taken into account because a lack of in-depth information. According to Lardon et al., (2009) biodiesel production from algae can potentially lead to abiotic depletion, acidification, eutrophication, ozone layer depletion, human and marine toxicity, ionizing radiations and photochemical oxidation [Lardon et al., 2009]. This indicates that the release of toxic components and environmental damage associated with biodiesel production from algae should be a point of focus for future research.



**Figure 4:** Overview of the most commonly used production process for biodiesel from microalgae. In and outputs associated with the different production stages are shown. The production of construction materials is not shown in this figure although this is a major factor in the sustainability, adapted from Chowdhury et al., 2012.

#### Life cycle analyses

A life cycle analysis (LCA) is a tool to assess the environmental impacts associated with all the different stages in the entire lifecycle of a product, from cradle to grave (or well to pump). This could include e.g. energy, construction materials, nutrients, GHGs and water. The criteria being used depend on the author and do often differ between studies. An LCA also requires a functional unit which can be e.g. 1 kg of biomass produced, 1 MJ of algae derived energy or 1 production year. This also depends on the



author. Furthermore many LCAs have system boundaries e.g. only steps which contribute at least 5% of the total energy use are taken into account [Sander & Murthy, 2010] this can neglect serious environmental impacts like the release of toxic compounds. This all has led to a wide variety of different LCA studies being published which are difficult to compare (**table 9**). One consistent, uniform, protocol for LCAs should be used. [Grierson & Strezov., 2012]. In **Figure 4** an LCA overview with its boundaries is presented for the most common used biodiesel production method (**Figure 4**).

**table 9:** Overview of differences between LCA studies. Adapted from Grierson & Strezov, 2012.

Criteria	System boundaries	Functional unit	Authors
NER, GHG balance, co-products	Strain to pump	1 year	Batan et al., 2010
NER, GHG balance, water consumption, co-products	Cultivation to conversion	10 GJ of biodiesel	Bretner et al., 2011
GWP	Cultivation to combustion	km driven	Campbell et al., 2010
GHG balance	Cultivation to extraction	MJ/ton biomass	Chisti, 2008
Energy use, land-use, water consumption, CO <sub>2</sub> , PO <sub>4</sub> <sup>-</sup>	Cultivation to processing	317 GJ of derived energy	Clarens et al., 2010
NER	Cultivation to processing	1 kg dry weight	Jorquera et al., 2010
GWP, eutrophication, acidification, toxicity, land-use	Cradle to combustion	1 MJ fuel combusted	Lardon et al., 2009
Relative mass flows	Cultivation to pump	1000 MJ of energy	Sander & Murthy, 2010
GWP, fossil energy consumption, co-products	Cultivation to combustion	1 ton biodiesel	Stephenson et al., 2010a
GWP, eutrophication, acidification, ozone depletion	Cultivation to pump (in 5, 10 and 20 years)	3650 kg of algae over 20 years	Soratana & Landis, 2011
Nutrient usage, water consumption	Cultivation to finished product	1 kg biodiesel product	Yanq et al., 2011

### Net energy ratio

The net energy rate (NER) is the total amount of (fossil) energy consumed/energy produced. Batan et al., (2010) state that fossil diesel has an NER of 0.19 while biodiesel from soy has a rate of 1.67 and algae based biodiesel a rate of 0.93. The highest energy sinks were the operation of the PBR and the oil extraction stage (by using hexane). [Batan et al., 2010] According to this LCA algae based biodiesel production would be energetically sustainable, however the production of nutrients and materials are not taken into account and CO<sub>2</sub> is provided without (energy)costs. These assumptions are unrealistic as these are often major contributors to the energy consumption. Brentner et al., (2011) did include those in and outputs and found an NER for the best case scenario of 1.08, which could turn sustainable with future research. Nutrient production and PBR operation were the largest energy sinks [Brentner et al., 2011]. Another study from Clarens et al., (2010) compared the NER of algae cultivation with the cultivation of corn, canola and switchgrass and found an NER of 0.95 for algae compared to NERs of respectively 0.12, 0.22 and 0.09. In this study conversion was not taken into account although they did take nutrient and CO<sub>2</sub> production into account (**table 10**). [Clarens et al., 2010]

De Boer et al., (2012) compared multiple LCAs and found that in most cases the cultivation in the PBR (including nutrient production) was the highest energy sink but also dewatering in some other studies

(table 10) [de Boer et al., 2012]. In the case of dewatering it turned out that centrifugation requires almost twice as much energy as filtration [Sander & Murthy, 2010]. Sevingé Itoiz et al., (2012) compared the energy requirements of vertical-column PBRs placed indoors (without daylight) and outdoors in Spain. The reactor indoor was artificially illuminated and kept at a constant temperature of 20 °C. The energy costs for keeping a constant temperature were about 2/3 of the total energy consumption while the illumination costs accounted for 1/6. Both indoor and outdoor PBRs were energy consuming, but the indoor PBR consumed roughly 100 times more MJ than it produced. [Sevingé Itoiz et al., 2012] This indicates that the placement of PBRs is an important aspect considering the NER, especially in colder climates like in the Netherlands. As this indoor PBR did not receive any daylight the placement within a greenhouse would lower the electricity requirements although the construction of the greenhouse would contribute substantial to the NER. To lower the energy requirements for future indoor cultivation in the Netherlands a greenhouse should be heated by waste heat [Baliga & Powers, 2010]. Biodiesel production from microalgae has currently no or only a small net energy gain. More research will be needed to make algae cultivation less energy intensive to be competitive with other crop fuels. Research should be focussed on the construction and operation of the PBR and on nutrient production.

**table 10:** Major energy consumption in different studies. Adapted from de Boer et al., 2012.

Cultivation	Harvesting	Extraction	Major energy cons.	Authors
PBR	Centrifugation	Solvent	PBR and extraction	Batan et al., 2010
PBR	Flocculation	Methanol	PBR	Brentner et al., 2011
PBR + open pond	Filtration	Bead mill	PBR and extraction	Razon & Tan, 2011
PBR + open pond	Filtration or centrifugation	Solvent	Harvesting	Sander & Murthy, 2010
PBR	Flocculation	Solvent	Cultivation in PBR	Stephenson et al., 2010a

### Greenhouse gas balance

The greenhouse gas (GHG) balance indicates how much greenhouse gasses (e.g. CO<sub>2</sub>, Methane (CH<sub>4</sub>) and NO<sub>x</sub>) are emitted per functional unit (often in CO<sub>2</sub> equivalents). Another used measure for this is global warming potential (GWP) [Soratana & Landis, 2011]. The GHG balance is dependent on the energy balance as with the production of energy GHGs are emitted. The common thought is that algae will capture CO<sub>2</sub> for growth and the CO<sub>2</sub> is released in the atmosphere again when the fuel is burned. However the production of construction materials, nutrients and electricity required for the entire process will emit GHGs as well. Soratana & Landis (2011) compared 20 PBRs made of different materials, with different life-spans and with or without the use of flue gas and waste streams. They concluded that the PBR construction material contributes more to the GWP than nutrient production does, making PBR life time an important factor. The best construction material considering GWP is glass although high-density polyethylene scored better on other environmental impacts. A PBR made of glass at a life time of 20 years which uses CO<sub>2</sub> from flue gas and nutrient from waste streams avoids almost 2 kg of CO<sub>2</sub> eq./kg algae produced although they did not include further downstream processing. [Soratana & Landis, 2011] For the production of 1MJ of algae biodiesel in a plastic bag PBR, 75 g of GHGs (primarily CO<sub>2</sub>) were avoided. This amount is larger than for soy biodiesel as N in closed PBRs cannot react with air, which otherwise leads to N<sub>2</sub>O emissions. [Batan et al., 2010]

Another study from Stephenson et al., (2010a) found an opposite result i.e. that almost 12 kg of GHGs were released per kg algae based biodiesel produced, which corresponds to 1.36 kg/MJ. This is a huge difference which is mainly caused by the intensive pumping needed for the tubular PBR design of Stephenson et al., (2010a) but also because no displacement of fossil fuels is taken into account [Stephenson et al., 2010a]. Clarens et al., (2010) also found that the cultivation of algae has a net emission

**table 11:** *NER & GHG balance of algae compared to other fuel crops.* Adapted from Clarens et al., 2010.

Fuel crop	NER	GHG balance (kg CO <sub>2</sub> eq./GJ)
Switchgrass	0.09	-76
Corn	0.12	-82
Canola	0.22	-50
Microalgae	0.95	+57

of GHGs i.e. 57 kg CO<sub>2</sub> eq./GJ. While with the cultivation of corn, canola and switchgrass respectively 82, 50 and 76 kg CO<sub>2</sub> eq./GJ are being captured (**table 11**) [Clarens et al., 2010]. Sander & Murthy (2010) compared the CO<sub>2</sub> emissions for filtration and centrifugation and found 60% higher emissions with centrifugation [Sander & Murthy, 2010]. All these differences and inconsistencies in LCAs make it hard to determine precise GHG balance of different cultivation designs. The GHG balance of biodiesel production from microalgae can be minimized by lowering the fossil fuels input associated with the production of primary electricity, heat, nutrients and construction materials. For example algae based biodiesel generated in China, operating on carbon-based electricity, will have a less favourable GHG balance compared to algae based biodiesel generated in Brazil or France (operating with less carbon-based electricity) [Shirvani et al., 2011]. Currently it seems that biodiesel production from microalgae has a net emission of GHGs. With more research a GHG reduction could be achieved, research should be focussed on nutrient production, PBR construction and the use of sea/waste water and flue gas.

#### *Freshwater consumption*

Biodiesel production from algae requires large amounts of freshwater, especially open pond cultivation consumes a lot of water due to evaporation. Not many research has been conducted on the water consumption of PBR systems, perhaps because it is often believed that no water loss will take place in a closed PBR. However this will be unlikely as PBRs (especially in warmer climates) need cooling, some water will be lost during drying and extraction and the water within the system cannot be used endless because of eutrophication, acidification and the increase of toxic compounds [Carlozzi et al., 2006; Mehlitz, 2009; DOE, 2010; Soratana & Landis, 2011]. Lardon et al., (2009) state that an open pond should be flushed every two months due these problems. The problems with toxicity for marine organisms are much higher with algae based biofuels compared to fossil fuels and other biofuels. This study does not state however which compounds become toxic, only that the problem can be lowered by not using pesticides and lower fertilizer usage. [Lardon et al., 2009] Currently a large human health risk assessment of algae production systems is taken place which focuses on toxins, toxic compounds, volatile organic carbon compounds (VOCs), metal accumulation and pathogenic organisms. Results are expected end 2012. [Sullivan, 2011]

Yang et al (2011) calculated that by using an open pond the production of 1 l biodiesel in the U.S. requires an input of 3726 l of freshwater. 85% of this amount is being lost in the process of harvesting, the rest is lost by evaporation, drying and a small amount with extraction [Yang et al., 2011]. Biodiesel production with this level of water usage would not be sustainable. Wigmosta et al., (2011) found a water consumption of 1421 l per l biodiesel (in open ponds) [Wigmosta et al., 2011]. The harvested water can be relatively easy recycled. With water recycling 84% less fresh water will be needed, when sea or wastewater is being used this could even decrease to 90%. This will lead to a water consumption of roughly 373 l freshwater per l biodiesel (**table 12**). [Yang et al., 2011] Baliga & Powers (2010) found a water consumption of 4-7 l/l biodiesel in a closed PBR, with most water lost in the feedstock production phase although they do not go into further detail [Baliga & Powers, 2010]. These studies do not give a

clear estimate for possible water consumption of a PBR in the Netherlands. The water consumption of algae based biodiesel production with a PBR in the Netherlands will be of a lesser problem as much of the water can be recycled and not much cooling is needed. The Netherlands are located at sea making seawater easily available although environmental risks should be studied thoroughly. The water consumption of algae based biodiesel is in all cases lower than that of other biofuels from crops, especially compared to other crops used for biodiesel production (**table 12**) [Gerbens-Leenes et al., 2009; Yang et al., 2011].

### *Nutrient usage*

Another important aspect regarding the sustainability of biodiesel production is nutrient usage. The most used nutrients are N and P, followed by other macronutrients including Na, S, Mg and K. Next to these they also require micronutrients like Fe, Cl, Ca and Bo and in a lesser extent Mn, Zn, Cu, Mo and Co. Diatoms also require large quantities of additional Silica. [WUR, 2011a] The production of these nutrient costs large amounts of energy and emits substantial amounts of GHGs. Furthermore, according to Soratana & Landis (2011) the production of nutrients also leads to the acidification, release of carcinogenics, eutrophication, ozone depletion, ecotoxicity and smog formation [Soratana & Landis, 2011]. The higher the algae biomass productivity the higher the nutrient requirements will be. Miller (2010) compared the nitrogen requirements of different biofuel crops and found that algae require the largest amount of nitrogen per GJ biodiesel produced after rapeseed and soybeans. The

**table 12:** *freshwater consumption of different fuel crops.* Only the use of freshwater is given. \* by using sea/waste water and water recycling. Adapted from Gerbens-Leenes et al., 2009. [Gerbens-Leenes et al., 2009; Baliga & Powers, 2010; Yang et al., 2011; Wigmosta et al., 2011].

<b>Feedstock</b>	<b>Fresh water consumption</b>
<b>Ethanol</b>	<b>(l water/l biofuel)</b>
Cassava	420
Sugar beet	822
Maize	1013
Potato	1078
Sugar cane	1364
Paddy rice	1641
Rye	1846
Barley	2083
Wheat	2873
Sorghum	4254
<b>Biodiesel</b>	
Microalgae PBR	4-7
Microalgae min* open pond	350-373
Microalgae open pond	1421-3726
Soybean	7521
Rapeseed	8487
Jatropha	11636

other crops required less than half the nitrogen, although Miller noted that for crops in practice often larger amounts are used because crops are not 100% efficient in N uptake (**table 13**). [Miller, 2010]

Chowdhury et al., (2012) compared their findings on nutrient usage of algae based biodiesel with other studies, but here again the results are highly diverse due to different LCA boundaries and allocation methods (**table 14**). Their own results (38 kg N and 5.3 kg P per 1000 kg biomass) show that nutrient usage is relatively low, this was achieved by using a 65% recycling factor of all nutrients after anaerobic digestion. Recycling of nutrients can be efficient as the lipids extracted do not contain N and P. [Chowdhury et al., 2012] Nutrient usage can be lowered by making use of a (natural) nutrient depletion at the end of the cultivation phase, which will lead to higher lipid content as well [Lardon et al., 2009]. The relative high nutrient usage calculated by Yang et al., (2011) do not include any recycling. When water is recycled the nutrient usage will be reduced by 55%, when sea or wastewater is used no nutrients need to be added except for P [Yang et al., 2011].

#### *Co-product allocation*

The production of biodiesel from algae could also yield different co-products. An important method to make the entire production process more sustainable is co-product allocation. This means that co-products can replace other processes or products which otherwise would lead to e.g. increase GHG emissions or energy consumption. There are multiple forms of co-product allocation e.g. direct allocation and economic allocation [Grierson & Strezov 2012]. Direct allocation takes place within the system

e.g. methane produced can be used to generate heat needed in other parts of the process (e.g. drying). But with this allocation method valuable co-products are of no use in the process, here economic allocation can be used to offset environmental impacts associated when the product is produced somewhere else. [Stephenson et al., 2010a] Glycerol is an important co-product but is of little value as it is also produced as a waste product from large scale soap production. Chowdhury et al., (2012) used glycerol to generate additional biodiesel through heterotrophic cultivation. [Chowdhury et al., 2012] Other co-products generated can include: proteins, non-fuel lipids and carbohydrates. Many of these will not be attractive for economic co-product allocation because of the enormous amounts produced if biodiesel would become a large scale operation. Therefore the allocation of the biomass residue to anaerobic digestion for methane production is the most promising option, while other output should be recycled as much as possible (**Figure 4**). [DOE, 2010]

**table 13:** *Nitrogen usage of different fuel crops.* Adapted from Miller, 2010

Fuel crop	N intensity (gr/GJ energy)
Willow	90
Sugar cane	110
Birch	160
Poplar	160
Miscanthus	210
Switchgrass	300
Oil palm	440
Sugar beet	460
Corn	490
Sorghum	1000
Microalgae	1100
Rapeseed	1400
Soybean	3900

**table 14:** *Nutrient usage reported by different authors.* Low and High are different nutrient regimes within the study. Adapted from Chowdhury et al., 2012.

	kg/1000 kg biomass (40% lipid)
<b>N</b>	38; 10.9 (Low) 46 (High) 23.6; 63.7; 132; 147
<b>P</b>	5.3; 2.4 (Low) 9.9 (High) 9.97; 20; 93

## *Outlook*

Compared to 1<sup>st</sup> and 2<sup>nd</sup> generation biofuels, algae based biodiesel is the only biofuel which could be sustainably developed in the future [Ahmed et al., 2011]. There are many aspects regarding sustainability, if biodiesel from algae is going to take place on a large scale the impacts of all these aspects should be minimized. The biggest risks for the sustainability of the process of biodiesel production are a high NER and large GHG emissions compared to other fuel crops. But there are many opportunities as well. Here the best options are presented: All the equipment should be constructed with materials which have long life-spans and are produced with using minimum amounts of (fossil) energy [Soratana & Landis, 2011]. The process should be as energy efficient as possible, which will also leads to lower GHG emissions. This can be accomplished by carefully choosing production designs and using “green” energy, but also by making using waste heat. Wastewater or seawater should be used to be less dependent on nutrients specially produced and to minimize fresh water consumption. All water should be recycled as much as possible. The biomass residue left after oil extraction should be used to generate methane with anaerobic digestion. Methane can then be used to generate heat or electricity. The remaining biomass after anaerobic digestion should be used as nutrients for the system, or when wastewater provides sufficient nutrients it can be allocated as fertilizer for agriculture or used for co-firing in power plants. To further lower nutrient usage, and to facilitate nutrient recycling, algae should be grown until the nutrients in the culture are depleted. Furthermore CO<sub>2</sub> should come from flue gas, avoiding CO<sub>2</sub> emissions from a power plant. The placement of the algae production facility is an important aspect. The facility should be located near a power plant to use flue gas but also waste heat. Currently in the Netherlands greenhouses already make use of waste heat from power plants. The facility should also be located or close to the sea (for using seawater) or at a location where wastewater can easily be obtained.



## 4. Challenges in scaling-up

### *Introduction*

Currently the production of algae based biodiesel has not been undertaken on a large commercial scale [Demirbas & Demirbas, 2011]. Despite the large amount of (promising) biodiesel related studies the commercial production still needs to be established. What are the major obstacles in the realization of commercial biodiesel production and what possible solutions are available? The cultivation of microalgae to date consist primarily of the production of valuable chemicals or as food and feed. In 2004 globally 5000 tons of microalgae biomass was produced, at high costs, for these purposes. The average price for 1 kg of algae biomass was €250, this price is way too high considering commercial biodiesel production as purpose. [Pulz & Gross, 2004]

### *U.S. aquatic species program*

The first major research project on microalgae for biodiesel production, the U.S. aquatic species program, was ended in 1996 after 18 years when the funding (\$25 million in total) was stopped. The goal of this project was to develop renewable transportation fuels from algae, with the main focus on biodiesel produced from microalgae (green algae and diatoms) with high lipid contents grown in open ponds, while using CO<sub>2</sub> from power plants. Their main findings are shown in the table below (**table 15**). [Sheehan et al., 1998]

**table 15:** Main findings U.S. aquatic species program. [Sheehan et al., 1998]

#### **U.S. aquatic species program - Main findings**

- Green algae and diatoms are most promising
- Nutrient starvation does not lead to increased oil productivity
- Maximum annual biomass productivity 182500 kg/ha/yr
- Low temperatures were a major limitation for high productivity
- Production costs in best scenario are twice as high as for normal diesel
- Biological factors (like max light utilization rate) responsible for high costs
- Little prospects for any alternatives to open ponds

In this project 3000 algae species were collected from which 300 were further investigated after more in depth screening. Most of these interesting species were green algae and diatoms. Nutrient starvation did increase lipid content per cell but at the same time slowed down cell division leading to lower overall oil productivities. This program was first to isolate and over-express a gen (for the production of ACCase) which was believed to increase lipid production. Tests although, gave no positive results. 90% CO<sub>2</sub> sequestration from flue gas was possible, but no perfect (i.e. with high growth + high lipid content) microalgae was found. The maximum annual production, calculated from a single day maximum, was high. But real biodiesel production would be much lower because of conversion, low temperatures at night, climatic variability and in a lesser extent by pond contamination with invading species.

Furthermore they concluded that because biodiesel is a low-value product not many other production designs are likely (i.e. PBRs). Thereby the main factors influencing costs are biological e.g. max light utilization rate, lipid content and growth rates. When an imaginary microalgae (i.e. with high light utilization rate) was used, biodiesel turned out to be (only) twice as expensive to produce compared to

normal (fossil) diesel, at that time. This is in a way a promising result as the price of a barrel of crude oil in 1996 was around \$30, compared to the price of roughly \$90 today. They also concluded that the resources land and water are no problem. [Sheehan et al., 1998]

### *Limitations*

The main limitation to the productivity is the amount of sunlight which can be captured. PBR designs are restricted to small diameters to allow sufficient sunlight penetration. With the placement of PBRs it is important to put the PBRs in such a position that shadowing of each other is minimized. For this reason it is unlikely for PBRs to have biodiesel productivities (l/ha/yr) of magnitudes higher than open ponds. Flat plate PBRs have the largest problems with shading. With scaling-up tubular PBRs also problems with CO<sub>2</sub>, O<sub>2</sub> and pH gradients are encountered while extensive pumping is required as well. Vertical column PBRs could be made higher but then the mixing rate would decrease and stability problems could occur. The only way to increase the diameter of a PBR seems to be by using an internal light source. This could be an LED illumination what would lead to increased costs and energy use. Another option would internal optical fibres connected to a light capturing structure which would not increase energy use but is expensive. [Janssen et al., 2002]

### *Productivity claims*

The lipid content of microalgae has been the major characteristic for research. Microalgae are said to be able to contain lipid levels of up to 70% [Metting, 1996]. This extreme high number is however not supported by clear measurements. Lipid levels of 20-50% are quite common [Chisti, 2007]. With the daily maximum production reached in the U.S. aquatic species program a lipid content of 50% will result in a maximum biodiesel yield of roughly 90 000 kg/ha/yr [Sheehan et al., 1998]. Wigmosta et al (2011) calculated a maximum biodiesel production in open ponds in the U.S., based on a maximum solar utilization rate of 10%, of ~110 000 l/ha/yr at Northern latitudes and even a ~150 000 l/ha/yr on latitudes closer to the equator. A survey was made to examine what area could possibly be available in the U.S. suitable for algae cultivation. It turned out that 5.5% of the total surface area of the U.S. would be available. When all this area would be used to produce biodiesel, the U.S. could produce 220 billion litres of biodiesel annually, equal to 48% of their petroleum import for transport. This would require 1425 l freshwater/l biodiesel. [Wigmosta et al., 2011] At this time this productivity claims are unrealistic, especially for open ponds. Even if these productivities could be met a huge amount of surface area is required while it would not even replace half of the imported petroleum for transport.

According to Waltz (2009) an oil production of 55 000 l/ha/yr can be accomplished with near-term technologies. However it is not stated which production system would be used. [Waltz, 2009] Others say that a realistic oil productivity would be around 40 000 l/ha/yr (in open ponds) [Biello et al., 2011] to 60 000 l/ha/yr (not mentioning which production system) [Savage, 2011]. Wijffels & Barbosa (2010) also used the same level of biodiesel productivity of 40 000 l/ha/yr (not mentioning which production system) [Wijffels & Barbosa, 2010]. These claims seem to be plausible for biodiesel production as they are all in the same range, although these estimates seem to be based on open ponds.

There are also others which claim to produce significant larger amounts of biodiesel. One company, Valant Products, in Vancouver, Canada claims that their PBR system has the potential to produce 935 000 l biodiesel/ha/yr. This seems unrealistic as this is five times the theoretical maximum amount of



sunlight received on a hectare. [Waltz, 2009] Another company, Joule Unlimited, expects to start commercial biodiesel production of 140 000 l/ha/yr with engineered cyanobacteria in 2012 [Savage, 2011]. Weyer et al., (2010) calculated a theoretical maximum production of 354 000 l unrefined oil/ha/yr for PBRs. According to these authors a current best case scenario would yield a maximum of 53 000 l/ha/yr. [Weyer et al., 2010] A maximum biomass productivity in the South of Spain would be 280 000 kg/ha/yr, while in the Netherlands this would be around 140 000 kg/ha/yr. However actual biomass production would be 30 000 kg/ha/yr in an open pond and 50 000 kg/ha/yr in a tubular PBR. [WUR, 2011b] An overview of these productivity claims is given in **table 16**.

**table 16: Productivity claims.** Microalgal oil productivity claims reported by different authors based on different cultivation methods and/or assumptions. \* 1 kg biodiesel = 37 MJ, 1 l biodiesel = 33-35 MJ, 1 kg  $\approx$  1.09 l [EU, 2009; Murthy, 2011]. \*\* X = cultivation method is not mentioned or not relevant which can be the case for theoretical maximum productivities.

Oil productivity	Measure*	Remarks	Method**	Author
30 000	kg/ha/yr	realistic NL	Open ponds	WUR, 2010b
40 000	l/ha/yr	realistic	Open ponds	Biello et al., 2011
40 000	l/ha/yr	realistic	X	Wijffels & Barbosa, 2010
50 000	kg/ha/yr	realistic NL	PBR	WUR, 2010b
53 000	l/ha/yr	best case scenario	X	Weyer et al., 2010
55 000	l/ha/yr	with near-term technologies	X	Waltz, 2009
60 000	l/ha/yr	realistic	X	Savage et al., 2011
90 000	kg/ha/yr	max based on single day prod.	Open ponds	Sheehan et al., 1998
110 000	kg/ha/yr	theoretical max high latitudes	Open ponds	Wigmosta et al., 2011
140 000	kg/ha/yr	theoretical max NL	X	WUR, 2010b
140 000	l/ha/yr	Joule Unlimited claim	PBR	Savage et al., 2011
150 000	kg/ha/yr	theoretical max low latitudes	Open ponds	Wigmosta et al., 2011
280 000	kg/ha/yr	theoretical max South of Spain	X	WUR, 2010b
354 000	l/ha/yr	theoretical max	X	Weyer et al., 2010
935 000	l/ha/yr	Valant products claim	PBR	Waltz, 2009

Today the largest PBR is located in Kötze, Germany (**Figure 5**). This plant consists of 20 tubular PBRs with a combined length of 500 km and a volume of 600 000 litres located inside a greenhouse and covers a surface area of 1200 hectares. The annual production is estimated to be 150 000 kg of biomass (*Chlorella vulgaris*) per year, which is only 125 kg biomass/ha/yr. This biomass is only used for food, cosmetics and medicines. [Janssen et al., 2003; Ullman et al., 2007]

Biello (2011) says that with a surface area of 2.7 million ha (roughly the size of Maryland) all transport fuels in the U.S. could be replaced by algal biodiesel [Biello, 2011]. To replace all transport fuels in Europe a surface area of 9.25 million ha (almost the size of Portugal) would be required [Wijffels & Barbosa, 2010]. A calculation, assuming that 1 l of biodiesel contains 34 MJ of energy [EU, 2009; Murthy, 2011] and a biodiesel productivity of 50 000 l/ha/yr [WUR, 2010b], shows that if the Netherlands want to meet a 14% share of renewable energy (only algae biodiesel) of the TPES (total primary energy supply) an area of nearly 276 000 ha (about the size of the province Zuid-Holland) would be required for the production. This would be impossible. However, it is unrealistic to assume biodiesel from microalgae alone will satisfy the 14% share. Currently only 4% of the TPES is derived from renewable energy sources (mainly from biomass and wind) [CBS, 2010]. For microalgal biodiesel

to give a fair contribution to this share (e.g. 1% share of the TPES) a surface area of around 20 000 (20 km<sup>2</sup>) would be required. This indicates that biodiesel from microalgae has potential to give a substantial contribution to the Dutch usage of renewable energy sources. The Netherlands however has too little surface area available to become a large producer of biodiesel from microalgae. Offshore algae cultivation might be an alternative.



**Figure 5:** *World's largest PBR.* This tubular PBR used for the production of *Chlorella vulgaris* for food, cosmetics and medicines is located inside a greenhouse situated in Kötze, Germany. Picture taken from [www.researchalgae.com](http://www.researchalgae.com).

### *Costs*

The production costs depend on the production method. A quick calculation in Waltz's (2009) article gives a production cost of \$2.6-\$5.3 per litre for open ponds and \$5.3-\$10.6 by using PBRs. The costs could be lowered to a minimum of \$1.6 per litre in the next 5 years. The production of biodiesel should be as simple as possible, for this reason production in a PBR will not be possible. [Waltz, 2009] According to Alabi et al., (2009) the costs for 1 litre of algae oil produced in a open pond, in a PBR, or by heterotrophic cultivation in a closed reactor are respectively \$14.44, \$24.60 and \$2.58. As a reference the costs to produce 1 litre of canola are \$0.88. [Alabi et al., 2009] The production by heterotrophic cultivation leads to higher productivities resulting in lower production costs. Kovacevic & Wesseler (2010) compared social costs (GHGs, food prices impact, fertilizer usage and supply security) of algal biodiesel with fossil fuels and found social costs to be more than 3 times higher for algal biodiesel due to nutrient input and lack of infrastructure. When CO<sub>2</sub> emissions can be traded at more than €100 per ton CO<sub>2</sub> algae biodiesel might become competitive (price of 1 ton of CO<sub>2</sub> on 26.03.2012 was €14 [Cozijnsen, 2012]). [Kovacevic & Wesseler, 2010] In the Netherlands there are subsidies available to make biodiesel production more economic attractive [EL&I, 2012]. Another possible way to make biodiesel production more economic viable could be by producing valuable co-products.

### *Commercialization*

The promises of biodiesel companies have attracted large investments. Some of the most important companies (+ received investments) specialized in genetic engineering are: Solazyme (\$70 million), Sapphire energy (\$100 million) and Synthetic Genomics (\$600 million) [Waltz, 2009; Biello, 2011]. These investments will most likely lead to progressions towards lower production costs. Especially with the huge investment in Synthetic Genomics the creation of an ideal organism for biodiesel production might be near. Next to genome sequestering their main focus is oil secretion to eliminate the costs

associated with intensive harvesting, cell disruption and extraction. [Waltz, 2009] In 2008 Solazyme has supplied more than 50 000 l of (heterotrophic produced) biodiesel to the U.S. navy with a production cost of \$112 per litre [Biello, 2011]. Now, in 2012, they have a new contract for 570 000 l while they are hoping to be able to sell algal oil to refineries by 2013 [Savage, 2011]. There are also cases where other companies, claiming to be able to commercial produce biodiesel, had to shut down despite large investments. A recent study funded by BP concluded biodiesel production from algae is far from economic viable unless it would be combined with wastewater treatment and the production of valuable co-products. [Savage, 2011]

## 5. Discussion

### Overview

The aim of this thesis was to give an overview of the process of biodiesel production from microalgae and its possibilities in the Netherlands. Microalgae strain selection, process technology, sustainability of the process and the challenges in scaling-up were discussed. Here the future of biodiesel production from microalgae in the Netherlands is discussed. This thesis was only focused on biodiesel produced from photoautotrophic microalgae grown in closed photo-bioreactors (PBRs) in the Netherlands.

### Limitations

In this thesis a small selection of seven species of microalgae has been made for determining the most suitable species for biodiesel production in the Netherlands. There are many more species known to have potential for biodiesel production. These species include e.g. *Skeletonema* sp., *Scenedesmus* sp. and *Schizochytrium* sp. [Chisti, 2007; Ahmed et al., 2011]. If the selection in this thesis would have been larger some other species might proved to be more suitable for production in the Netherlands. A review of all potential species for biodiesel production would take large efforts however it would be very useful for finding the most suitable microalgae for a particular location and process design. From a total of about 50 000 species of microalgae only a relatively small proportion has been investigated for their potential in biodiesel production [Mata et al., 2010]. Because there are many species yet to be discovered there is a potential that some of these species are useful for biodiesel production.

Two species of microalgae especially *N. gaditana* but also *C. vulgaris*, turned out to be the most suitable species for biodiesel production in the Netherlands according to the MCA with the corresponding criteria used in this thesis. Another species, *B. braunii*, had a better overall score but does not produce lipids suitable for biodiesel production. However this species does produce large amounts of hydrocarbons which can be used to produce gasoline or jet fuel [Sakamoto et al., 2012]. This also could be a good alternative to biodiesel production. More research will be needed. The functioning of the algae determines the biodiesel productivity but also the costs and sustainability of the production process. Therefore the selection of a microalgae is an important aspect which is dependent on many factors. In this report I have chosen five factors: lipid productivity, nutrient usage, climatic suitability, content of valuable co-products and harvesting ease. I believe these factors are most important for successful biodiesel production in the Netherlands. But there are obviously more factors which influence the performance of microalgae. Some of these factors are linked to the process design while others are primarily biological. Important factors considering process design are: resistance to shear stress which is important when there is strong mixing inside the PBR which is often the case with tubular PBRs, resistance to large pH gradients caused by the amount of dissolved CO<sub>2</sub> which mainly occurs in long tubular PBRs and performance under different light-dark cycles caused by mixing and size of PBR. Some other important biological factors are: the light utilization efficiency, resistance to seasonal variety, reaction to nutrient depletion and the exact composition of lipids. [Rodolfi et al., 2009] Before the realization of a biodiesel production plant all factors should be taken into account. When this is done another species might turn out to be more suitable for biodiesel production in the Netherlands.

This thesis focuses only on the use of photoautotrophic organisms (i.e. which use sunlight and CO<sub>2</sub> for growth). Microalgae grown under heterotrophic conditions have been shown to produce higher amounts of biomass and lipids per litre [Miao & Wu, 2006]. These productivities are independent of sunlight. This has a big influence on the design of the bioreactor. The bioreactor does not need to be shaped to optimize light capturing. With this cultivation method more volume of bioreactor can be fitted per area resulting in even higher productivities per surface area. Regarding the problems with land-use in the Netherlands this option seems preferable. However more research will be needed especially regarding the sustainability of this cultivation method as no atmospheric CO<sub>2</sub> is being captured and additional resources (e.g. sugars) are required to feed the microalgae.

Furthermore this thesis is limited to the analysis of only 3 different types of PBRs. These are the three main basic designs (flat plate, tubular and vertical-column PBRs). There are also hybrid systems which combine e.g. a vertical bubble column with a tubular part as external light harvesting unit. Some of the PBRs can be placed in an open pond to have better control over the temperature. [Singh & Sharma, 2012] PBRs placed in an open pond can also only consist of plastic bags which would lower the costs of construction materials substantial, although the life span would be shorter as well [Wijffels & Barbosa, 2010]. Before deciding which PBR to use in the Netherlands these other designs should also be considered and compared.

After cultivation in the PBR there are different production pathways which eventually lead to biodiesel. The first step, harvesting, can be done on different ways than the 3 basics methods (sedimentation, centrifugation and filtration) compared in this study. These basic methods can be further divided in sub methods like normal filtration and ultra-filtration or chamber press and belt filtering [Grima et al., 2003; Brennan & Owede, 2010]. Harvesting could even become superfluous as the extraction of lipids would become possible from wet biomass for example by using enzymes or when lipids would be secreted [Radakovits et al., 2010]. Subsequent phases of the production process, extraction and conversion, may both be done by a large variety of methods. A review and comparison of these methods is outside the scope of this thesis, but will be necessary before realization.

Biodiesel from microalgae is an active research topic with many articles being published. Most of these articles however, especially older ones, are focused on open pond cultivation. Most of the articles focused on closed PBRs are based on lab experiments with controlled parameters. Only a few studies actually focus on larger scale (outdoor) PBRs. Lab experiments often turn out to be (over) optimistic because weather conditions are not taken into account. The realization of a large scale PBR based on lab experiments has gone wrong before [Waltz, 2009]. Also only one study focuses on microalgae cultivation in PBRs in colder climates [Baliga & Powers, 2010]. More research will be needed in this area. Another problem is the lack of consistency in the literature. Too many different standards are being used making good comparisons difficult. The productivity of algae can be measured as dry or wet biomass and as lipid productivity, from which the latter is favourable. These measurements are then measured in different units e.g. as kg or litres per area or volume. This problem is even more present with LCA studies. As stated in chapter 3 the functional units and system boundaries differ greatly between studies which make results harder to interpret.

### SWOT analysis

To give better insights in the future of algae based biodiesel production in the Netherlands a SWOT analysis was performed (**Figure 6**). In this SWOT analysis microalgae biodiesel production in the Netherlands is compared to 1<sup>st</sup> and 2<sup>nd</sup> generation biofuels in general. Although there are large differences between and within those categories of biofuels [Miller, 2010; Gerbens-Leenes et al., 2009], the use of algae for biofuels (3<sup>rd</sup> generation biofuels) differs in most cases clearly from the other generations biofuels. The criteria used in this SWOT are: productivity (1), costs (2) and the sustainability (3) of the process.

### Strengths

The cultivation of microalgae lead to much higher productivities per hectare compared to other fuel crops. Due to these high productivities less surface area is required for comparable yields of other fuel crops. Microalgae, in contrast to 1<sup>st</sup> generation fuel crops, can be cultivated on soil unsuitable for food production. Many microalgae contain valuable co-products which can contribute to a more economic viable process. The residual biomass can be used to generate electricity by anaerobic digestion to lower the need for external electricity and thus contribute to the costs and sustainability of the process. Furthermore no pesticides need to be used if algae are cultivated in a closed PBR.

		Helpful	Harmful
		Strengths	Weaknesses
Internal		<ul style="list-style-type: none"> <li>-High productivities (1)</li> <li>-Can be grown on land unsuitable for food production (2,3)</li> <li>-Content of valuable co-products (2)</li> <li>-Biomass residue is suitable for anaerobic digestion (2,3)</li> <li>-Low land-use (2,3)</li> <li>-No need for pesticides (2,3)</li> </ul>	<ul style="list-style-type: none"> <li>-Elaborate (PBR) construction (2,3)</li> <li>-Energy intensive operation (2,3)</li> <li>-High nutrient requirements (2,3)</li> <li>-Environmental damage (1,2,3)</li> <li>-Average water requirements (2,3)</li> </ul>
		Opportunities	Threats
External		<ul style="list-style-type: none"> <li>-Use of flue gas (2,3)</li> <li>-Use of sea/waste water (2,3)</li> <li>-Use of waste heat (2,3)</li> <li>-Recycling of water/nutrients (3)</li> <li>-More efficient harvesting and extraction methods (1,2,3)</li> <li>-Genetic engineering (1,2,3)</li> <li>-Increasing prices of CO<sub>2</sub> for CO<sub>2</sub> trade (2)</li> <li>-Dutch policy favours sustainable energy sources (2)</li> <li>-Climate change (1,2)</li> <li>-Decreasing public support for other forms of "Green Energy" (2)</li> </ul>	<ul style="list-style-type: none"> <li>-Climatic unsuitability (1,2,3)</li> <li>-Limited space available (2,3)</li> <li>-Development of other sustainable energy sources (2)</li> <li>-Unknown negative impacts (1,2,3)</li> </ul>

**Figure 6:** SWOT analysis for biodiesel production from microalgae in the Netherlands. The results of a SWOT (Strengths, Weaknesses, Opportunities and Threats) analysis are given. Microalgal biodiesel production is compared to the production of 1<sup>st</sup> and 2<sup>nd</sup> generation biofuels. Criteria used: productivity (1), costs (2) and the sustainability (3).

### *Weaknesses*

The weaknesses “elaborate (PBR) construction”, “energy intensive operation” and “high nutrient requirements” all lead to high costs but also to a lower sustainability. This is mainly caused by high GHG emissions associated with the production of respectively construction materials, energy and nutrients. These weaknesses also lead to a higher NER. The weakness “elaborate (PBR) construction” could be turned into a strength by using other and less construction materials. Materials with a long lifespan should be used which can be produced in an environmental friendly and cost effective way. The use of recycled materials might be an option. The energy intensity of the process could be lowered by more research on efficiency of the production process. The fossil energy use could be lowered by using energy generated within the system (i.e. by anaerobic digestion) and by using energy from other sources i.e. solar or wind energy. This could turn the weakness “energy intensive operation” into a strength. “High nutrient requirements” could be, relatively easy, turned into a strength by using waste or seawater and flue gas (for CO<sub>2</sub>) while recycling the residual biomass for nutrients. The use of waste or seawater would also turn the weakness “average water requirements” into the strength “low water requirements”. The total and precise environmental damage resulting from the production of microalgae biodiesel is not yet fully clear. Possible net GHG emissions and high NER but also possible accumulation of metals, production of toxins and acidification will contribute to environmental damage and will have a negative impact on the process. To turn this weakness into a strength first more research will be needed to realize adequate measures. For example water might need to be treated before discharge but possibly also extra policies might be required.

### *Opportunities*

To turn the opportunities “use of flue gas” and “use of sea/waste water” into strengths a microalgae strain should be selected which is able to reach high productivities while grown with these resources. The production system should be located near a power plant for the use of flue gas and at the same time near the sea or a source of (agricultural) wastewater. It might be necessary to treat flue gas or wastewater before it can be used, which would increase costs. In the Netherlands microalgae cultivation might require additional heating due to low temperatures. By using waste heat from industries the need of specially produced heat can be decreased, decreasing costs and increasing sustainability. After oil extraction and anaerobic digestion the nutrients in the residual biomass need to be recycled. The largest amount of water which need to be recycled is the water normally lost by harvesting. To make sure this water and biomass residue are suitable for recycling the upstream use of toxic compounds should be minimized or additional treatment will be necessary. By more efficient harvesting and extraction methods biodiesel productivity, costs and the sustainability could be increased. Big opportunities for biodiesel production from microalgae lay within the possibilities of genetic engineering. With genetic engineering for example lipid yields could be increased substantial, microalgae could be engineered to secrete lipids or to be more resistant to different forms of stress. This will require multiple years of research but will make significant contributions to the success of microalgal biodiesel. Other opportunities to make microalgal biodiesel more economic viable are possible increasing prices of CO<sub>2</sub> for CO<sub>2</sub> trade but also because the Dutch policy favours sustainable energy sources which could result in (additional) subsidies. Ongoing climate change also brings opportunities as higher temperatures might increase the productivity although the effect of climate change on hours of sunshine is unknown. Also climate change could increase the public support for biodiesel production from algae once it has proven to be a truly sustainable energy source. Also the

public support for other sources of “green energy” like wind energy or 1<sup>st</sup> and 2<sup>nd</sup> generation fuel crops could decrease in favour of microalgae biodiesel.

### *Threats*

The best way to turn the threats “climatic unsuitability” and “limited space” into strengths would be by moving the production process to another country. This indicates that biodiesel production on a large scale is unlikely in the Netherlands. However a strain of microalgae might be found (or genetically engineered) which would be able to reach high productivities with the Dutch climate. The problem of limited space could be solved by moving algae cultivation to offshore locations however much research will be needed. The development of another, more sustainable and less expensive, energy source will be at the expense of the success of microalgal biodiesel. Furthermore there might be, at this time, unknown negative impacts. This also occurred with the realization of other renewable energy sources. For example the production of biodiesel from soy turned out to be a driver of deforestation in the Amazon, Brazil [Lima et al., 2011]. Another example are wind turbines which turned out to be a threat to birdlife by direct mortality (collision) but also by habitat loss (by visual intrusion and disturbance) [Drewitt & Langston, 2006].

Currently the weaknesses of biodiesel production from microalgae in the Netherlands outweigh the strengths. The main reasons are the net GHG emissions, a high NER and the high costs associated with the PBR construction, nutrient production and energy requirements. However the opportunities give promising prospects for the future. With more research microalgae have the potential to become a better source of energy compared to other fuel crops. Based on this SWOT analysis a more detailed sceptical and optimistic view on the future of biodiesel production from microalgae in the Netherlands is given.

### *Sceptical view*

Microalgae require a larger nutrient input for the same amount of energy derived compared to 2<sup>nd</sup> generation biofuels [Miller, 2010]. When algae cultivation would not be combined with the treatment of wastewater and flue gas biodiesel production from algae cannot become sustainable. Especially when looking at the NER and GHG balance because the production of nutrients and CO<sub>2</sub> are energy intensive while large amounts of GHGs are emitted. All the biomass residue should be recycled to re-use the nutrients to lower the NER and amounts of GHGs being emitted. This could result in a build-up of metals and other (toxic) compounds in the algae. Also all water should be recycled which limits harvesting techniques (like the use of flocculants) as these chemicals will be hard to remove and could have an adverse effect on growth when water gets contaminated [Borrowitzka & Moheimani, 2010]. More research is needed on the effects of algae cultivation on the water quality but probably treatment will be needed to minimize environmental impacts before used water can be discharged. Another possible problem with high productivities is the production of growth inhibiting substances by the microalgae themselves, however much is unknown about this process [Richmond, 2000].

With an average annual temperature of 10.1 °C and 1639 hours of sun [KNMI, 2010] the Dutch climate is not ideal for microalgae cultivation. As a comparison the U.S. government used the criteria of temperatures  $\geq 13$  °C and  $\geq 2800$  hours of sun for suitable algae cultivation site selection [DOE, 2010]. These low light conditions and low temperatures lead to requirement of additional heating and preferably also additional illumination. The easiest way to do this is probably by placing the PBRs



inside greenhouses. This will further increase energy requirements but also increase the costs of the PBR. Without the placement within a greenhouse, the construction and operational costs will already be too high for profitable biodiesel production. Another problem is the land requirements of algae cultivation, also in the case of PBRs. A biodiesel productivity of 50 000 l/ha/yr may become possible with PBRs in the Netherlands. In 2010 the total amount of biofuels being used in the Netherlands was 399 million litres from which biodiesel accounted for 121 million litres [CBS, 2010]. For biodiesel from microalgae to give a substantial contribution to the biodiesel use multiple km<sup>2</sup> would be required for the cultivation. With genetic engineering a more efficient microalgae might be developed to increase lipid productivity and lower nutrient requirements. However the genetic engineering of microalgae to increase biodiesel production is still in its infancy [Radakovits et al., 2010].

### *Optimistic view*

Biodiesel from microalgae is considered as one of the most promising biofuels. The ability of microalgae to capture atmospheric CO<sub>2</sub> greatly exceeds that of terrestrial plants while not competing for arable land with food crops. The oil productivities (l/ha/yr) of algae are also much higher than those of other fuel crops [Miller, 2010]. These large amounts of algae produced require large amounts of nutrients to grow. In the Netherlands algae cultivation could take place near the sea and use seawater. This will minimize freshwater consumption but also decreases the need of additional nutrients (only P need to be added). This would make microalgae the most sustainable fuel crop only looking at nutrient consumption [Miller, 2010; Yang et al., 2011]. Another nutrient source could be (agricultural) wastewater. Some microalgae species are able to grow in this water further reducing nutrient requirements. The residual biomass can be used to generate methane through anaerobic digestion to generate electricity and afterwards this residue could be recycled for nutrients. Another option could be co-firing this residue in a (adjacent) power plant. Microalgae also need carbon for growth, CO<sub>2</sub> can be supplied to the culture via flue gas from power plants [Vunjak-Novakovic et al., 2005]. This eliminates the need for specially produced CO<sub>2</sub> and at the same time reduces CO<sub>2</sub> emissions from the power plant. As with the cultivation of microalgae CO<sub>2</sub> is being captured, carbon credits can be sold. The current value of a ton CO<sub>2</sub> is relatively low but possible higher prices in the future could give a substantial contribution to the economic viability of the production process.

PBRs should be made from materials which have a long lifespan, this contributes to the sustainability. As the construction and operational costs of PBRs are high for such a low value product as biodiesel. The price of biodiesel from microalgae will be high for the first years. But there are indications that a combination of improved biological productivity and fully integrated production systems can bring the cost down to a point where biodiesel from microalgae can compete with petroleum at approximately \$100 per barrel [DOE, 2010]. The Dutch government has made a fund (SDE+) of 1.7 billion euro in 2012 to stimulate the production of sustainable energy sources by paying the difference between the production costs of normal energy and of sustainable energy [EL&I, 2012]. In the future biodiesel productivities of above 100 000 l/ha/yr might be reached. With these productivities a surface area of 40 km<sup>2</sup> would be enough to have a 4% share of the TPES (in 2010). However much research will be needed to reach these productivities. Current productivities (around 50 000 l/ha/yr) are already much higher compared to other fuel crops, indicating microalgae are a more favourable energy source. But even if higher productivities could be reached, the Netherlands cannot become a major microalgae biodiesel producer due to limitations in space. It does, however, have the potential to gain much knowledge about algae cultivation which can be used as a source for other countries and for the

production of cultivation systems [Hamm & Wijffels, 2012]. To gain knowledge while working towards commercial biodiesel production the first PBRs should be constructed aiming at producing valuable chemicals from microalgae. Recent advances present opportunities to develop the process of biodiesel production from microalgae in a sustainable and economical way within the next 10 to 15 years [Wijffels & Barbosa, 2010]. Especially breakthroughs in the field of genetic engineering (e.g. the secretion of oils) could lead to big steps towards sustainable and economic viable biodiesel production from microalgae. But still much research is needed.

### *Conclusions*

The question in this thesis was: Which process to produce biodiesel from algae will be most suitable, looking at strain selection, process technology and sustainability? At this time the SWOT analysis turns out primarily negative for biodiesel production from algae in PBRs in the Netherlands. However the opportunities are very promising and indicate that biodiesel from microalgae has a larger potential compared to 1<sup>st</sup> and 2<sup>nd</sup> generation biofuels to be commercially and sustainably produced in the Netherlands in the future. But it can be concluded that the Netherlands cannot become a large producer of biodiesel from microalgae. The main reason is that the production will always require a large surface area, even with PBRs, which is not available in the Netherlands. It is neither likely that biodiesel production from photoautotrophic microalgae in PBRs is going to take place on a commercial scale for national consumption in the Netherlands in a near future. The costs to efficiently cultivate microalgae in a PBR in the Netherlands are too high for the production of biodiesel. Microalgae are a promising source for biodiesel which can be produced (very) sustainably provided that cultivation uses sea/waste water, flue gas and waste heat while both water and nutrients are recycled and the residual biomass is used to generate energy through anaerobic digestion. Much research will be needed to optimize this system and to get better insights on possible environmental damage.

If biodiesel would be produced from microalgae it is important to keep the following aspects in mind. The selection of which microalgae to use is important. At this time there is no perfect microalgae species. From the selection in this thesis *N. gaditana* and *C. vulgaris* turned out to be most suitable for biodiesel production in the Netherlands. However some big breakthroughs in the field of genetic engineering are required before the realization of commercial biodiesel production. A vertical column PBR from where biomass is harvested through centrifugation or filtration (based on the species of microalgae) is the most promising production option from the selection in this thesis. However the choice of the PBR design and which harvesting technique to use are important but are not as crucial to the success of the system as e.g. the incorporation of using sea/waste water and flue gas while recycling water and nutrients. Furthermore extraction and conversion are both important steps in the production process where major savings of energy, costs and GHGs can be accomplished.

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