Microalgae cultivation systems

Analysis of microalgae cultivation systems and LCA for biodiesel production



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Master thesis Sustainable Development – Energy and Resources Utrecht University

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Summary

The purpose of this research is to analyse three different cultivation systems for microalgae cultivation. Microalgae have been considered as a promising alternative for the sustainable production of energy and materials, for their high possible photosynthetic efficiency, high lipid content (i.e., 30-70%), low land use, no competition for crop areas, high ability to fix nitrogen and phosphorus, and the consumption of CO₂. One of the most important products of microalgae is biodiesel that is able to, with co-production of other products such as nutrients, replace fossil feedstocks.

Different systems are available for microalgae cultivation: open ponds, and tubular and flat panel photobioreactors. Open ponds are easy and cheap to exploit on large scale for mass production of microalgal biomass, but are less suitable for the production of specific strains or products due to little control of reaction conditions. Tubular and flat panel photobioreactors allow for much better control of these conditions, and allow for using higher concentrations resulting in a higher productivity.

Factors that influence microalgae growth are the temperature, solar irradiation intensity, reactor geometry, concentration, and the availability of CO_2 and nutrients. To assess the growth rate and productivity of the algae a growth model was developed. This model assessed the microalgae growth affected by the amount of solar irradiance, the transmission through the wall, and the conversion efficiency of the algae which depends on the algae strain and environmental conditions such as pH and nutrient availability. Using Nannochloropsis sp. in Dutch conditions, the productivity was $64 \text{ g/m}^2/\text{day}$, which is a slight overestimation when compared to literature. One of the weaknesses of the model is that it does not consider the reactor geometry, meaning no possibility to specify for open pond, tubular or flat panel, and that it is linear, therefore not considering feedback mechanisms or interconnections of the parameters and effects.

A second model was developed based on 6 sub models: light input, transmission, shading, light gradient inside the reactor, temperature and growth of the microalgae. Light input is influenced by the orientation of the reactor, the location and seasonal and daily changes. When multiple parallel reactors are used, the height and distance between them determine the shading. Transmission through the reactor wall is determined by the wall material being for example glass or plastic. Inside reactor the concentration and light path determine the amount of light the algae receive. Temperature fluctuates throughout the year and the day, but must be close to a optimal temperature and below the lethal temperature of the specific algae. The ability of the microalgae to utilize the light efficiency reflected in a maximum growth rate, respiration rate and the chlorofyll-carbon ratio in the cell, ultimately determines the growth rate of the microalgae.

The model was used to analyse the volumetric and areal productivity of open ponds, horizontal and vertically stacked tubular reactors and horizontal and vertical flat panels. This was done for two algae strains: Chlorella a fresh water algae, and Nannochloropsis a saline strain, and for two locations Rotterdam (NI) and Narbonne (Fr). The analysis was done for one year and for one day every season to allow for comparison of seasonal differences and annual productivity. In terms of volumetric productivity the horizontal tubular reactors are the most advantageous (0.8-1.3 kg/m³/day). Per unit of area the flat panel is more beneficial (59-75 g/m²/day). The model reported a high performance in open ponds, that is not very plausible according to literature, probably due to the assumption of optimal conditions and too high concentrations. Working with the model showed that it is difficult to design one integrated model for all reactor configurations: the model was accurate for closed photobioreactors, but overestimated for open ponds. However, this models does allows for clear comparison of different locations or algae strains.

The next step was to use these results for a life cycle analysis of a base case for biodiesel production. The process consists of five steps: growth, harvesting of the algae (incrasing the mass percentage to 40%), pre treatment to disrupt the cells and further dry the slurry, extraction of the oil from the cells by using chloroform/methanol and transesterification of the lipids into oil and ultimately biodiesel.

The LCA was performed considering 1 kg of biodiesel produced. The non-renewable energy use and greenhouse gas emissions as well as land use are considered. The results were allocated according to mass and economic value. The life cycle analysis showed that a horizontal tubular reactor with Nannochloropsis in Rotterdam is the least energy and emissions intensive out of the assessed configurations. A large share of the energy consumption and greenhouse gas emissions is caused by the nutrients provided to the algae, therefore finding a different source for these such as waste water, could be a way of reducing the impacts of microalgae cultivation. This case was used for further analysis of the production of biodiesel.

The analysis of a base case for the production of biodiesel showed that the drying and purification processes are the most energy intensive steps. The total non-renewable energy use of the production of biodiesel from microalgae biomass is 3.29 times higher than the energy content of the biodiesel produced and 280 gram of CO₂-eq was emitted. When allocated to mass or economic value, considering the fact that biodiesel is not the only product that is extracted from the algae, the energy consumption is 0.95 or 1.34 MJ/MJ biodiesel produced respectively and emissions are 81-114 g CO₂-eq. This type of allocation shows that besides finding less energy intensive alternatives for the drying and purification it is also very important to consider co-production of multiple (high-value) products from microalgae in order to make the process more energy efficient and lower the emissions.

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1. Introduction

Microalgae have been considered as a promising alternative for the sustainable production of energy and materials (Wijffels & Barbosa, 2010; Singh & Olsen, 2011). Some characteristics that cause this high potential of microalgae are: high possible photosynthetic efficiency, high lipid content (i.e., 30-70%), low land use, no competition for crop areas, high ability to fix nitrogen and phosphorus, and the consumption of CO₂ (Wijffels R. H., 2007; IEA Bioenergy, 2010; Chisti & Yan, 2011). These special characteristics make microalgae biomass a suitable raw material for the production of biodiesel (Scott *et al.*, 2010) as well as for the replacement of feed stocks for the chemical industry (Wijffels R. H., 2007; IEA Bioenergy, 2010). However, more research and technological advances are needed on in order to take advantage of this potential.

One of the most important possible products from microalgae are biofuels which could replace fossil fuels for the transportation sector which in turn represent a share of 61% of the total oil consumption (International Energy Agency, 2010). In this respect an alternative fuel could reduce the burden that fossil fuel use causes on the environment in terms of greenhouse gas emissions, while slowing down the depletion of resources, and diminish the reliance on imports and their associated political constraints. Since microalgae have the ability to produce large amount of fatty acids (e.g. 25-35% for Chlorella sp. and up to 60% for Nannochloropsis sp.) that can be converted into biodiesel, microalgae seem to be an important opportunity to help meeting the global demand for transportation fuels by means of this production from biomass unlike any other form of oil crops (Nigam & Singh, 2011; Chisty, 2007).

Microalgae technologies for biofuels production are still under development and consequently they are not commercially available yet, and causing high estimations of production costs (Norsker, Barbosa, Vermuë, & Wijffels, 2011). Thus, more research on algae strains, cultivation and harvesting methods, extraction technologies, and ultimately scale-up, are necessary to reduce the production costs. In fact, it has been stated that biodiesel production from microalgae will only be feasible when other products like bulk chemicals, materials, food and feed are simultaneously produced meaning that the full potential of the microalgae must be used (Wijffels & Barbosa, 2010). The following sections describe the potential of microalgae for energy and nutrients production linked to the concept of biorefinery.

Energy and nutrients production from microalgae

Fossil fuels represent around 80% of the current energy consumption and they are also used as feedstock for nutrients production. This causes not only high dependability of these resources and the countries they come from, also depletion of resources and the emission of greenhouse gasses. These environmental and geopolitical concerns are good reasons to explore other feedstocks for materials that offer these possibilities in a more independent and sustainable way.

Biomass is one of the feedstocks that can offer these possibilities. Biomass has been classified into first, second and third generation. First generation means using sugar, grains or seeds, but has the disadvantage of competing with food production and leading to an increase in the cost of crops. Second generation means lignocellulosic biomass from agricultural waste or non-edible crops that is converted by thermochemical processes such as Fisher-Tropsch, enzymatic hydrolysis or anaerobic digestion. These fuels have the advantage of less competition with food production and since they are produced for energy purposes only, land use efficiencies are higher (Singh Nigam & Singh, 2011)

Third generation biomass does not compete with food production and also does not put a burden on land use. This can be for example microbes and microalgae. Yeast, fungi and microalgae have the ability to produce large amount of fatty acids that can be converted into biodiesel (Singh Nigam & Singh, 2011). Together with this production of biodiesel, microalgae produce a wide variety of useful products that can replace in some cases fossil fuels as a feedstock for chemical industry.

The Concept of Biorefinery

A biorefinery must try to optimize the value of every fraction in the feedstock, the same way a petroleum refinery does. This can maximize profits and material efficiency and is in line with the principles of green engineering: integration of available energy and material flows (Foley, Beach, & Zimmerman, 2011). The concept of biorefinery includes a spectrum of possibilities. There are different possibilities to use biomass in the production of chemicals. The first option is a 'green biorefinery'. 'Green' refers to the nature of the feedstock. It can be grass, clover or lucerne that is grown for preservation of the landscape. A 'whole-crop biorefinery' means that the feedstock, which can be wheat or maize are separated in straw and for example corn. The straw is used as a carbohydrate and the corn can be converted by polymerization, chemical modification or biotechnological conversion into biomolecules.

The last important option is 'lignocellulose-feedstock biorefinery'. The feedstock is naturally lignocellulose from straw, reed, grass, wood, paper waste, etc. It seems to be the most promising large-

scale biorefinery because of optimal feedstock, similar products as the traditional petrochemical industry and low feedstock costs (Kamm & Kamm, 2004). In the following section the route from feedstock, via platform chemicals to a range of products will be described to give an overview of the possibilities of a biorefinery.

Feedstock

The term feedstock refers to the raw materials used in biorefinery. To be able to replace hydrocarbons such as natural gas and oil, the elements carbon and hydrogen, oxygen, nitrogen and some metals need to be present. This includes a large set of molecules that is suitable for the production of chemicals. There are several ways of dividing them into groups. The first and most important is on a molecular basis. This separates hydrocarbon and lignin feedstock from triglycerides and proteins. The sources for biorefinery can also be divided by precursors like wood, soya, sugar beet and grass or by the way of growing them; in a dedicated crop or are they residues from agriculture, forestry and industrial activities.



Platform chemicals

Figure 1 - Biobased products (Kamm & Kamm, 2004)

Production of bulk chemicals is based on a number of platform molecules, which are the basis of a wide range of products. Biorefinery will be based on a different set of platform molecules than currently used. The chemical structure of biomass forces the industry to choose different ways of producing chemicals. Although the traditional chemical platform molecules can also be derived from biomass, that will result in higher costs and lower yields. Therefore it is more beneficial to choose new strategies. A list of most promising bPM's (bio-Platform Molecules) was proposed for further research by the US Department of Energy in 2004 and updated in 2007 (Werpy, T.; Petersen, G. (eds), 2004; Holladay, J.E.; Bozell, J.J.; White, J.F.; Johnson, D., 2007). An overview of bPM's based on sugars is shown in figure 2.

This results in a wide range of chemicals to choose from in synthesizing products. Some general remarks are that the most favorable reaction is always the greenest. Additions are greener than substitutions and eliminations, because the atom economy is better. This means all atoms that are put in are used in the product of the reaction. Large amounts of solvents should also be avoided and catalysts should be used in every step (Clark & Deswarte, 2008)

Bio-platform molecules have much higher oxygen content in comparison to platform molecules from fossil resources (e.g., ethylene, benzene) (Nikolau, Perera, Brachova, & Shanks, 2008). Opposing to what normally occurs in the petrochemical industry: adding functionalities, there will be a shift to where a large part of the functionality is already present in the molecule. This makes it possible to avoid harsh and environmental damaging oxidation procedures and to use greener steps like reduction with hydrogen gas over a heterogeneous catalyst (Clark & Deswarte, 2008).



Figure 2 - Bio-based Platform Molecules: C3-C6 (Nikolau, Perera, Brachova, & Shanks, 2008; Werpy, T.; Petersen, G. (eds), 2004)

Products

As mentioned before, bio-refinery products can be divided into two categories: materials and energy. To replace the current use of fossil fuels, both need to be developed to a sufficient level. Often the production processes are integrated, because both can be made out of the same feedstock and some platforms have both functions e.g., hydrogen and bio-ethanol. In addition to that, co-production can make production processes economical attractive. For example, methane and hydrogen can be a byproduct of ethanol production from sugarcane and can be used to sell separately or to use in the same process to make more efficient (Hernandez & Kafarov, 2007).

The most important chemical and material products are the following (Cherubini, 2010):

- Chemicals (fine chemicals, building blocks, bulk chemicals)
- Organic acids (succinic, lactic, itaconic and other sugar derivatives)

- Polymers and resins (Polysaccharides, polyesters, polyurethanes, polyamides, phenol resins, furan resins) (Patel & Cranck, 2007)
- Biomaterials (wood panels, pulp, paper, cellulose)
- Food and animal feed
- Fertilizers

Microalgae Biorefinery

A microalgae biorefinery follows to concept of every other type of biorefinery as described by the International Energy Agency for their Task 42 Biorefineries: 'Biorefining is the sustainable processing of biomass into a spectrum of marketable products and energy' (Jong, Higson, Walsh, & Wellisch, 2011).

The major advantages of using microalgae as a feedstock in a biorefinery are:

- Microalgae do not compete for resources with conventional agriculture since it can be cultivated in seawater or brackish water, on non-arable land, and utilizing waste streams for nutrients such as nitrogen and phosphorus.
- Microalgae use carbon dioxide emitted during combustion processes as a source of carbon. 1 kg of algae can take up 1.8 kg of CO₂ during the growth process.
- The biomass is homogeneous and can be used entirely instead of e.g. only seeds or roots, which results in a higher productivity than plant biomass has and it can be harvested during all seasons (Jong, Higson, Walsh, & Wellisch, 2011).
- Algal biomass contains no lignin, which is advantageous because the valuable carbohydrates do not have to be separated from the less-valuable lignin, which is often a complicated and resource intensive process (Foley, Beach, & Zimmerman, 2011).

Biorefinery of microalgae can produce two types of products: energy and materials. Energy from microalgae includes fuels, such as biodiesel, hydrogen and biogas. Materials vary from different kind of polymers to very specific pigments or antibiotics.

The co-production of products other than biodiesel from algae has great advantages. The biochemical composition as compared to lignocellulosic energy crops can offer new opportunities for co-products and offers a chance to utilize the microorganism as a whole (Foley, Beach, & Zimmerman, 2011). This can be very interesting from a cost perspective, since these co-products can add significant value to the production of biofuels from microalgae.

Biobased products

Algae consist just like plant biomass mainly of lipids, proteins, and carbohydrates. The ratios depend on the species and the growth conditions. Microalgae store energy in the form of hydrocarbons or lipids and can vary from 50% proteins to 50% lipids. Other valuable compounds include: pigments, antioxidants, fatty acids, vitamins, anti-fungal, -microbial, -viral toxins, and sterols.

This offers a wide spectrum of useful products. The non-polar lipids (triacylglyceride: TAG's) are mainly used for the production of fatty acid methyl esters (FAME), biodiesel and glycerol products. The polar fraction is used to produce for example omega 3, 6 or 9 fatty acids for nutritional purposes, but also to produce polymers or epoxides. The pigments and the sterols are a feedstock for food supplements, steroids and animal feed. Then there are the carbohydrates, such as starch or other glucans that can be directly converted to biofuels or bio plastics. Alginates and more complex polysaccharides are a source for food additives to work as a gelling agent or to absorb water in dehydrated products. The amino acids are an important source for nutrient recycling and are used animal feed. Finally, secondary metabolites and inorganics are a feedstock for antibiotics and for various natural products (Foley, Beach, & Zimmerman, 2011).

Downstream processing

To produce all these different chemicals and energy carriers, multiple steps in this downstream processing are required to isolate and convert the biomass feedstock into useful products. This starts with a choice in cultivation technology: an open pond that is a simple system that allows for relatively low cost scale up, or a photobioreactor that is more complicated and expensive but also offers the possibility of high levels of control of the growth conditions and therefore can provide higher yields of specific products (Brennan & Owende, 2010).

The next phase is the harvesting of the algae. The selection of the harvesting technique depends on the density, size and the value of the desired products from the algae. Generally the process of harvesting can be divided into two stages: bulk harvesting and thickening. Examples of bulk harvesting are flocculation, flotation and gravity sedimentation. This is used to increase the amount of solid matter to 5-15%. Thickening is done by filtration or centrifugation to further concentrate the slurry and is therefore a more energy consuming process (Brennan & Owende, 2010; Molina Grima, Belarbia, & Acién Fernández, Recovery of microalgal biomass and metabolites: process options and economics, 2003).

After the algae have been harvested, there are several possibilities for further processing of the biomass. In case of using it directly for energy production in the form of e.g. syngas, hydrogen or ethanol the biomass is thermochemical or biochemical conversed into one of these products. This includes gasification to produce syngas, direct combustion for electricity, anaerobic digestion for methane, fermentation into ethanol and photo biological hydrogen production (Brennan & Owende, 2010).

Otherwise the product needs to be extracted from the cells and conversed into useful products. Therefore the cell is disrupted and the product, such as the lipid fraction, dissolved. The methods for cell disruption and extraction include using sodium hydroxide, alkaline lysis or high-pressure homogenisation followed by solvent extraction or extraction with supercritical CO_2 . Solvent extraction with solvents such as hexane, ethanol, chloroform and diethyl ether are widely used to extract astaxanthin, β -carotene and essential fatty acids from various microalgae (Molina Grima, Belarbia, & Acién Fernández, 2003). Extraction and conversion can be combined into one process using sonification and direct transesterification or supercritical methanol extraction (Brennan & Owende, 2010; Brentner, Eckelman, & Zimmerman, 2011). Crude extracts are generally filtered and purified by various chromatographic methods to obtain the metabolite of interest. Proteins are usually purified using ion exchange chromatography (Molina Grima, Belarbia, & Acién Fernández, 2003). The last step is the conversion into useful products, in case of lipids this is done by esterification. (Brentner, Eckelman, & Zimmerman, 2011).





Background of the research

One of the first large research programs on microalgae was the Aquatic Species Program (ASP) funded by the US Department of Energy (Sheehan, Dunahay, Benemann, & Roessler, 1998). This program focused on the development of sustainable transportation fuels from microalgae biomass grown in open ponds by utilizing waste CO₂, but this program was ended in 1996 due to budget reduction. The most important advances were made regarding the understanding of the physiology and biochemistry of the algae, genetic engineering and manipulation of the algae metabolism. This led to demonstration projects on open pond systems, but the high cost remained as an obstacle (Sheehan, Dunahay, Benemann, & Roessler, 1998).

A more recent and currently active program on bioenergy and microalga is executed by the International Energy Agency (IEA). A working group focusses on bioenergy, structured in a nineteen tasks of which three are inclusively focused on the use of microalgae for energy purposes. Their research goals are oriented to the identification of the most cost intensive processing stages, savings of CO₂, and demonstrations required in developing commercial algal technologies (IEA Bioenergy, 2010). Next to these kind of large programs, many specific researches are carried out on e.g. algal species, design of production systems and assessment of regional opportunities by both academia and startup companies (Chisti & Yan, 2011; Demirbas M. F., 2011).

One of these more recent initiatives is the project this research is a part of, funded by Climate KIC (Knowledge Innovation Community) an innovation network that strives for pan-European discussion and on-going innovation by bringing academics, businesses, governments and NGO's together (Climate KIC, 2012). In this respect they have set up this project called: 'Biorefinery of microalgae: sustainable feedstock supply for integrated production chains'. The project is led by Wageningen University and Research Centre (WUR), who has extensive experience with research on microalgae, both fundamental and applied in the form of pilot projects and full-scale production facilities. The other academic partner is Utrecht University, who has wide experience in environmental assessment, LCA and process analysis and assessment, for biofuels and bio products. Next to these universities, business partners participate, striving for the creation and assessment of a new value-chain around the microalgae feedstock and a business plan and in the end a demonstration project.

Since the main objectives of the Biorefinery project are clearly set (i.e., exploration of biorefining options, determination of a researching strategy and further innovation of biofuels and other valuable products from algae), a microalgae growth model and a LCA study would be a very useful tool in reaching these goals. Modelling of the microalgae growth in different type of reactors and in varying circumstances can be the basis of a life cycle analysis provide insight in the environmental burden of all elements in the process of producing biodiesel and chemicals from microalgae and consequently help to determine a fruitful direction for further research and pilot projects.

Problem definition

However algae technology seems very promising, the actual potential of algal biorefinery is still unclear and therefore the way to commercialization of algal technologies. To develop competitive and sustainable processing routes for biorefinery of algae, more knowledge needs to be gained on the growth of microalgae, and on the environmental impacts of all stages in the process. This means that the relevance and sensitivity to many parameters such as for example for the cultivation stage: light input, reactor specifications, algae characteristics or growth regime needs to be described in order to assess the exact environmental impacts of microalgae biomass production, and the conversion into biodiesel.

Aim of the research

The aim of this research is to develop a model for microalgae growth that accounts for all relevant variable and effects such as the type of photobioreactor that is used, the orientation, location and materials of this reactor, the specific growth characteristics of the microalgae and effect of temperature changes. The results of this model can be used to perform a life cycle analysis on the production of biodiesel from microalgae. This results in the following research question:

What are the expected environmental impacts of a microalgae biorefinery system based on the analysis of different microalgae growth technologies?

The sub questions that will be addressed are:

- 1. What are the best available technologies for cultivation (open ponds, PBR)?
- 2. What are the best reported operational conditions for each reactor?
- 3. What does a reliable growth model according to each technology look like with respect to:
 - a. Location
 - b. Strain
- 4. What are the environmental impacts of a base case for biodiesel production?

This research will consist of two parts. The first step will be the development of a microalgae growth model based on the earlier work of Jonker (Jonker, 2010). This will be adapted and then compared to a kinetic model. The result will be a model for microalgae growth that can model microalgae productivity based on the specific irradiance, algae strain characteristics, cultivation system and temperature. This productivity data will be used in the second part of this research.

The second step, described in section 6, will be on the life cycle analysis of a base case of biodiesel production. The productivity data from the microalgae growth model in combination with data on the harvesting, extraction and conversion of microalgae into biodiesel will be put into an existing model to make an assessment of all environmental effects of the process.

2. Microalgae production

Microalgae are prokaryotic or eukaryotic microorganisms that range in size from 0.5. – 50 µm (Sheehan, Dunahay, Benemann, & Roessler, 1998). Microalgae grow photosynthetically; using light and carbon dioxide to produce oxygen and carbohydrates. Algae have been estimated to include anything from 30,000 to more than 1 million species. Guiry recently made a conservative estimate of 72,500 algal species of which 44,000 have probably been published (Guiry, 2012). Microalgae are grouped in classes based on shared biochemical and physical characteristics such as the presence and characteristics (length, number, hairs, point of insertion) of flagella, cell-wall composition and type of stored photosynthetic product (Sheehan, Dunahay, Benemann, & Roessler, 1998). Some very common classes of algae used for microalgae cultivation are (Sheehan, Dunahay, Benemann, & Roessler, 1998):

- Bacillariophyceae (diatoms)
- Charophyceae (stoneworts)
- Chlorophyceae or green algae such as Chlorella, Dunaliella, Scenedesmus, Haematococcus, Nannochloris
- Chrysophyceae (golden algae)
- Cyanobacteria or blue-green such as spirulina. These are eukaryotes, in contrast to all other microalgae species that are prokaryotes
- Dinophyceae (dinoflagellates)
- Phaeophyceae (brown algae)
- Rhodophyceae (red algae)
- Eustigmatophyceae such as Nannochloropsis,

In table 1 an overview is given of the biomass productivities and lipid content of a few very common microalgae species, differentiated by cultivation system.

Table 1 – Overview of productivities for different reactors and microalgae strains							
Type of	Strain	Productivity	Algae	Amount	Light	Source	
reactor		(g/l day)	conc.	of lipids	path		
			(g/l)		(cm)		
Open pond	Chlorella sp.	0.11-0.32	1-2	11-15%	0.6	(Doucha & Lívanský,	
						2006; Converti,	
						Casazza, Ortiz, Perego,	
						& Del Borghi, 2009)	
	Spirulina platensis	0.06-0.18	0.75-2	17%	7.8	(Pushparaj, Pelosi,	
						Tredici, Pinzani, &	
						Materassi, 1997)	
	Spirulina platensis	2.1	7.5	20%	30	(Jiménez, Cossío,	
						Labella, & Niell, 2003)	
Flat plate	Nannochloropsis	0.18-0.27	2.6	60%	10	(Cheng-Wu, Zmora,	
						Kopel, & Richmond,	
						2001)	
	Nannochloropsis	0.3-0.36	2	60%	4.5	(Rodolfi, et al., 2009)	
	Arthrospira	2.10	4	25%	1.5	(Pushparaj, Pelosi,	
	platensis					Tredici, Pinzani, &	
						Materassi, 1997)	
Horizontal	Nannochloropsis	0.16-0.73	5	12.7-	4.3	(Chini Zittelli, Lavista,	
tubular				21%		Bastianini, Rodolfi, &	
						Vincenzini, 1999)	

	Isochrysis galbana T-iso	0.076	1.5	20%	6.4	(Bergeijk, Salas- Leiton, & Cañavate, 2010)
	Isochrysis galbana	0.32	1.19	8 %	2.6	(Molina Grima, et al., 1994)
Vertical tubular	Haematococcus pluvialis	0.06	0.44	34%	20	(García-Malea López, et al., 2006)
	Phaeodactylum tricornutum	0.5	0.07	37%	19	(Sánchez Mirón, Cerón García, García Camachoa, Molina Grima, & Chisti, 2002)
	Chlorella sorokiniana	0.3-1.47	1.5-5	36-40%	3.8	(Ugwu, Ogbonna, & Tanaka, 2002)

The main variables and effects

Microalgae growth is a process that requires very specific reaction conditions to become optimal. The most important conditions are temperature, culture density, light input (depending on location and season, reactor type and specifications, mixing, degassing, and nutrient concentration (nitrogen and phosphorus)). Every strain also has certain specifics that affect their growth such as the light absorption coefficient (depending on the chlorophyll concentration in the cell) and the maximal specific growth rate.

Growth kinetics

Growth for microalgae means not growth in size of a single cell, but an increase in the number of cells. This population growth is measured by the cell density over time. Microalgae growth runs through a number of phases: the adaption or lag phase, an exponential growth phase where productivity increases, followed by a linear growth phase with constant productivity, a stationary phase and finally accelerated death. This is depicted in figure 4a. It can be observed that microalgae produce biomass during light hours, but loose a part of that during the night due to cell respiration.

Temperature

Microalgae often have an optimal temperature, which usually is a band of a few degrees between 10 and 40°C, where growth is most effective. With lower temperatures productivity declines rapidly and with higher temperatures the algae dies. Figure 4b shows an example of experimental data on the growth rate of four different microalgae strains affected by temperature change.



Figure 4 - (a) Phases in microalgae growth (Sánchez Mirón, Cerón García, García Camachoa, Molina Grima, & Chisti, 2002); (b) Microalgae growth and temperature for different strains (Dauta, Devaux, Piquemali, & Boumnich, 1990).

Light input

Light input is almost the most important variable in algae growth. Only a specific bandwidth: 400-700 nm is photosynthetically active radiation (PAR) and therefore useful to the algae. This is 43% of the incoming

radiation. Every strain has different abilities to use this radiation, depending on chlorophyll concentrations for example. Incoming radiation depends on the location of the reactor and the season.

Culture density

Culture density is closely correlated to microalgae growth. A higher concentration blocks algae cells from the incoming radiation if the light path is too long. Thin flat plate or tubular reactors can therefore use higher concentrations than open ponds.

Carbon dioxide

Microalgae use carbon dioxide and produce oxygen during their growth. Therefore it is important to provide sufficient CO_2 input, mixing and degassing in order to not limit the algae growth. For this CO_2 input industrial waste gasses can be used, for example from cement or ammonia production.

Nutrients

Microalgae use besides water and carbon dioxide for photosynthesis also nutrients for their growth and the production of certain specific fatty acids, pigments or vitamins. The most important nutrients are nitrogen and phosphorus usually in the form of nitrogen oxide, ammonia or urea and phosphorus oxide. These can be provided by using waste streams like waste water. This way, algae can also be part of the purification process of waste water. Nitrogen is an essential element in proteins and for the cell division. Lack of nitrogen results in a build-up of lipids in the cell. This can be an opportunity in biodiesel production. Phosphorus is also essential for almost all cellular processes of the algae. A limitation in phosphorus results in a lower growth rate. Similar to nitrogen starvation, a phosphorus deficiency causes lipid accumulation.

From some elements only very small amounts are needed for optimal growth. These are called trace nutrients. The most important elements are sulphur and magnesium for building amino acids and chlorophyll, and calcium for nitrogen metabolism. Other elements that are mentioned in literature for showing a direct physiological effect on algae growth are manganese, nickel, zinc, boron, vanadium, cobalt, copper and molybdenum (Lavens, P; Sorgeloos, P. (eds.), 1996).

Mixing

A well-mixed culture is important to keep the reaction conditions optimal; supply sufficient CO_2 and nutrients and remove the produced oxygen (by degassing). This can be done by using a mechanical mixing system such as a paddle wheel in an open pond or by maintaining a certain flow rate by supplying carbon dioxide in a tubular or flat plate system.

Growth technologies

Microalgae can be grown in an open system or in different type of closed photobioreactors. The choice for a certain type or reactor depends on the location, available space and water, cost and the desired products.

Open raceway pond

Open systems include unstirred shallow ponds, stirred circular ponds and paddlewheel stirred raceway ponds. Typical depth is 10-50 cm to allow for appropriate illumination of the culture. The size of the circular and raceway ponds is maximum $1.5-3 \times 10^4$ L. The extensive shallow ponds can be up to 1×10^9 L in size, which equals 250 ha (Borowitzka, 1999). An open system is sensitive for contamination, therefore is mainly used for the commercial production of algae strains with very specific growth conditions, such as high nutrient concentration. Examples are Chlorella sp., Spirinula sp. and Dunaliella salina (Jorquera, Kiperstok, Sales, Embiruçu, & Ghirardi, 2010).

The reason these open systems are so widely used in the commercial production of micro algae and cyanobacteria are mainly economical. Open ponds are relatively easy to maintain and scale up and have low cost compared to closed culture systems (Borowitzka, 1999).



Figure 5 - Microalgae cultivation systems: 1 - Flat plate in plastic bag, 2 - Open raceway pond, 3 - Horizontal tubular PBR, 4 - Vertically stacked horizontal tubular PBR (all pictures are taken at AlgaeParc Wageningen).

Closed systems

Closed culture systems for micro algae have some specific advantages over open systems. Firstly they enable the sunlight to be distributed over a larger surface area. Since the saturation efficiency (the illumination intensity at which the algae have their maximum growth efficiency) usually is 100-200 μ E/(m²s) while illumination on a sunny tropical day can be up to 2000 μ E/(m²s), much higher area efficiencies can be achieved. Because of the efficient use of illumination, higher cell densities can be used, meaning higher volumetric productivity. Other advantages of closed systems are that they have almost no evaporation and loss of CO₂, no risk for contamination and more control on growth conditions such as nutrient concentration (Postena & Schaubb, 2009; Pulz, 2001)

Tubular photobioreactors

Tubular photobioreactors are the first developed closed reactors for microalgae cultivation (Torzillo, et al., 1986). A tubular system consists of long horizontal tubes connected to form walls, horizontal or inclined panels or helices, sometimes covered by a greenhouse roof. Diameters are 10-60 millimetres; lengths can be up to several hundred metres. The main advantage is the possibility for good temperature control, enabling for the use of high concentrations and very high photosynthetic efficiencies compared to open ponds. A good size-length ratio is important to avoid oxygen build up at the end of the tube. Disadvantages are the energy needed for pumping and a gradient in CO₂, O₂ and pH (Torzillo, et al., 1986; Richmond, Boussiba, Vonshak, & Kopel, 1993).

Flat panel reactor

The most common design is the flat panel reactor, consisting of two glass sheets with a light path varying from a few millimetres to 10 centimetres (Posten, 2009). These flat reactors are connected in cascade facing the sun with the proper tilt angles to assure maximal exposure to direct irradiation (Hu, Guterman,

& Richmond, 1996). This can also be achieved by using plastic bags and a construction to keep them in vertically aligned walls (Morweiser, Kruse, Hankamer, & Posten, 2010). Typical productivities lie around 0.1-0.3 g/L/day. Panel reactors seem in principle more promising than the horizontal reactors, since they may be set at variable orientations aimed at maximal exposure to solar energy throughout the year. In addition, the dissolved oxygen path is only a few feet long in the vertical panel reactors, preventing O2 build-up (Hu, Guterman, & Richmond, 1996).

Column photobioreactors

Vertical columns include vertical bubble columns, usually used for indoor experiments. Diameters are over 20 cm, which implies a dark area in the middle of the column. To solve this, an annular column is formed, consisting of two cylinders of different size (e.g. 40 and 50 cm) to form a wrapped flat plate reactor. In the inside of this annular column lamps could be fitted to increase productivity (Borowitzka, 1999).

Table 2 - Characteristics of microalgae reactors							
Reactor	Size	Productivity	Advantages	Limitations	Source		
type							
Circular &	1.5-3 x	low	Relatively cheap, low	Little control of	(Borowitzka,		
raceway	10 ⁴ L		maintenance, low	culture conditions,	1999)		
ponds			energy input, good for	risk of			
			mass cultivation of	contamination,			
			algae	limited to a few algae			
				strains, poor			
				productivity, occupy			
	10.00			large land mass			
Tubular	10-60	fairly good	Large illumination	Gradients of pH,	(Mata,		
	mm x		surface area, suitable	dissolved oxygen and	Martins, &		
	several		for outdoor cultures,	CO_2 along the tubes,	Laetano,		
	munarea		naduativitiaa	of well growth	2009) (Ugwu,		
	111		rolativoly choan	of wall growth,	Auyagi, &		
			relatively cheap	space	2008)		
Flat nanel	Un to	good	Large illumination	Scale-un require	(11gw)		
i lat pallel	400 L	goou	surface area, suitable	many compartments	Aovagi, &		
	100 1		for outdoor cultures.	and support	Uchivama.		
			good for	materials. difficulty	2008)		
			immobilization of	in controlling culture	(Brennan &		
			algae, good light path,	temperature, some	Owende,		
			good biomass	degree of wall	2010)		
			productivities,	growth, possibility of			
			relatively cheap easy	hydrodynamic stress			
			to clean up, readily	to some algal strains			
			tempered, low oxygen				
			build-up				
Column	5-20 L	fairly good	Compact, high mass	Small illumination	(Brennan &		
photobio-			transfer, low energy	area, expensive,	Owende,		
reactor			consumption, good	shear stress,	2010)		
			mixing, easy to	sophisticated			
			sterilize, reduced	construction			
			photo inhibition and				
			photo-oxidation				

3. Generic model of algae growth in photobioreactors

This microalgae growth model is based on the work of Jonker (2010) and Zemke *et al.* (2008). The model is a simulation model based on parameters such as: irradiance, respiration, temperature, limitation by factors such as CO_2 and O_2 concentration, pH, nutrients and mixing. This model was rebuild and tested in order to assess if it was a model with sufficient level of sophistication and reliable results for further environmental analysis.

Model description

Three factors are important for determining microalgae production rate: solar irradiance, the efficiency of light transmission to microalgae and the efficiency of conversion of incident sunlight to biomass in microalgae (Zemke, Wood, & Dye, 2008). The formulas that are used in this section are from Zemke *et al.*, all symbols and values are given in table 3. A schematic overview of the model is given in figure 6.

The productivity (*P*) is determined by the solar irradiance, the efficiency at which the algae capture the solar energy, and the transmission efficiency. Since this will result in a productivity of energy per square metre this has to be divided by the energy content of the algae.

$$P = \frac{E_s \tau \varepsilon_a}{E_a} \tag{1}$$

The solar energy capture efficiency (ϵ_a) is defined by the (sub-optimal) environmental conditions, the efficiency of photon utilization, maximum photosynthetic efficiency and the respiration rate:

$$\varepsilon_a = \varepsilon_{env} \, \varepsilon_{ph} \, u_p \, (1-r) \tag{2}$$

The limiting effect of sub optimal environmental conditions (ϵ_{env}) is simply defined by the effect of the separate conditions:

$$\varepsilon_{env} = F_{mix} F_{CO2} F_{Nutr} F_{pH} F_{O2} \tag{3}$$

Algae are not evenly productive at all light intensities: a higher photon flux density (PFD) results in a lower biomass yield per mole of photons (Janssen, Tramper, Mur, & Wijffels, 2003). This model has included that by using the Bush Equation as proposed by (Zemke P. E., 2010) for the efficiency of photon utilization:

$$Up = \left\{ \begin{pmatrix} \frac{ls}{li} \end{pmatrix} * \left(Ln \left(\frac{li}{ls} \right) + 1 \right) Ii \ge ls \\ 1 & li \le ls \end{pmatrix}$$
(4)

According to this equation productivities decrease with higher light intensities, but only from the saturation intensity of 200 μ mole m⁻² h⁻¹ (Thimijan & Heins, 1983). Below this saturation intensity photon utilization efficiency equals one. Assumed for this model is that the PFD is constant at, or below the saturation intensity so that there is no loss in efficiency because of high light intensities.

The transmission efficiency (τ) of sunlight to algae is determined by the optical light distribution, the light absorption coefficient of the microalgae strain, the area efficiency and the amount of photosynthetically active radiation:

$$\tau = \varepsilon_{opt} \alpha \eta \, cPAR \tag{5}$$

The model from Jonker also included a factor for water temperature, based on (Sukenik, Levy, Levy, Falkowski, & Dubinsky, 1991):

- Water temperature
$$(T_w) = 0.9 * (T_{ave} - T_{ampl} * COS (2\pi * 1/24))$$
 (6)

(7)

- Factor water temperature $(F_{Tw}) = (-.00003^*T_w^3) + (.026^*T_w) + 1.0446$

According to (Sukenik, Levy, Levy, Falkowski, & Dubinsky, 1991) the water temperature (equation 6) has an effect on the maximum photosynthesis rate following a sine function. For equation 7 no source or clear derivation was given, and since it multiplies the productivity with 1.3, the effect of this factor on the final results of the model was considered too large to use without a justification.

Table 3	Table 3 - List of abbreviations and values used in the simulation model						
Symbol		(Value) & Unit	Source				
α	Light absorption coefficient of	1	(Zemke, Wood, & Dye, 2008)				
	microalgae						
CPAR	Photosynthetic active radiation	43%	(Thimijan & Heins, 1983); (Janssen,				
			Tramper, Mur, & Wijffels, 2003)				
Ea	Energy content algae	26.2 MJ/kg	(Barbosa, et al., 2005)				
Es	Solar irradiance	MJ m ⁻² h ⁻¹	(SoDa, 2004)				
c	Sub ontimal environmental	_	(Zemke Wood & Dve 2008)				
Cenv	conditions		(Zenike, Wood, & Dye, 2000)				
ϵ_{opt}	Optical light distribution	96%	(Zemke, Wood, & Dye, 2008)				
ε _{ph}	Maximum photosynthetic efficiency	27%	(Zemke, Wood, & Dye, 2008)				
F _{CO2}	CO ₂	0.94	(Jonker, 2010)				
F_{mix}	Mixing	0.94	(Jonker, 2010)				
F_{Nutr}	Nutrients	0.95	(Jonker, 2010)				
F ₀₂	02	0.93	(Jonker, 2010)				
F_{pH}	рН	0.98	(Jonker, 2010)				
F_{Tw}	Factor water temperature	-	(Oliveira, Monteiro, Robs, & Leite,				
			1999); (Janssen, Tramper, Mur, &				
			Wijffels, 2003)				
II	photon flux density incident	MJ m ⁻² h ⁻¹	(Zemke P. E., 2010)				
	distributed inside the						
	photobioreactor						
Is	Saturation photon flux density of	0.158	(Thimijan & Heins, 1983)				
	microalgae	$MJ m^{-2} h^{-1}$	(200 µmole m ⁻² h ⁻¹ /4.57 µmole W ⁻¹ *				
			3600 s h ⁻¹ /				
			1 000 000 J MJ ⁻¹)				
η	Area efficiency	90%	(Zemke, Wood, & Dye, 2008)				
Р	Productivity	gm ⁻² h ⁻¹					
r	Respiration	0.1	(Sukenik, Levy, Levy, Falkowski, &				
			Dubinsky, 1991)				
Tampl	Temperature amplitude	°C					
T_{ave}	Average water temperature	°C					
T_w	Water temperature	°C	(Sukenik, Levy, Levy, Falkowski, &				
		0.4	Dubinsky, 1991)				
up	Saturation efficiency	%	(Zemke P. E., 2010)				



Figure 6 - Modelling scheme

Results and discussion

With the values as provided in Table 3, the results of the model are a microalgae production of 2.70 g m⁻² $h^{-1} = 64$ g m⁻² day⁻¹ by a solar irradiation of 12 MJ m⁻² day⁻¹, which is average for the North of France (SoDa, 2004). Global irradiance data can be found in Annex I.

Literature shows a productivity of 20 g m⁻² day⁻¹ for closed photobioreactors, only in case of very productive algae strains and ideal conditions (high illumination, very short light dark cycles) a productivity of over 50 g m⁻² day⁻¹ is achieved (Janssen, Tramper, Mur, & Wijffels, 2003; Lee, 2001).

Two main problems of this microalgae growth model are: the results seem to be higher that what can be expected based on literature, and it needs specific data on all of the parameters to become a model that can simulate algae growth in different photobioreactors and with different conditions. At this point mainly generic data is available hampering the user from specifying between different types of photobioreactors, experiment types and algae strains.

The fact that the microalgae productivity is so high according to this model is mainly due to the fact that only optimum values were available. These result in a production of 64 g/m^2 day, where a production of 20-40 g/m² day is more commonly found in literature (Zhang, Miyachi, & Kurano, 2001; Chini Zittelli, Rodolfi, Biondi, & Tredici, 2006; Eriksen, 2008). This result is also very sensitive to changes in the conditions. Since the model is a multiplication of factors, the result changes accordingly to a change in any of the input parameters.

Secondly the model is completely based on parameters such as a percentage representing the limiting effect of insufficient CO_2 availability or the amount of energy needed for respiration. Some of these characteristics are well known or can be calculated for different algae species or photobioreactors. This applies for area efficiency, optical light distribution, and respiration. Other characteristics are not specifically enough available. Especially effect of the sub optimal environmental factors is very unclear. According to Zemke there is no effect since the reactor is designed to be optimal regarding these factors

(Zemke, Wood, & Dye, 2008; Zemke P. E., 2010). In the research of Jonker this is assumed to be 2-6% (table 3) for each factor, adding up to a total of 23.5% reduction of the productivity, but without justification. Besides the high total number, this is too general and not applicable to different algae species, since for example some species are less sensitive to pH changes than others.

Finally, some factors, especially those related to light distribution, are represented in an incomplete manner. The light intensity that microalgae experience is determined by the light incident on the outside of the reactor, density of the mixture, length of the light path and mixing (Fernández, Camacho, Pérez, Sevilla, & Grima, 1997). These factors are not represented in this model or are not related e.g. mixing is only included as a percentage representing the limiting effect.

In a photobioreactor algae experience not a constant PFD due to mutual shading and liquid flows. At the outside of the reactor algae are exposed to too much light, especially in outdoor systems (12 MJ m⁻² day⁻¹ average in Europe, equals 1200 μ mole m⁻² h⁻¹). On the inside of the reactor light intensities are much lower, causing the productivity to increase significantly. Fast fluctuations in light intensity (micro-milliseconds) appear to increase photosynthetic efficiency whereas slower light/dark cycles (several seconds-tens of second's results in a lower productivity because of photoacclimation (Janssen, Tramper, Mur, & Wijffels, 2003).

Conclusion

In state of the art PBR's environmental conditions such as pH, temperature and nutrient availability are relatively easy to keep at an optimal level. If these factors are optimal, the culture becomes light limited. Light intensity, light/dark regimes, light path, cell density and dilution are the main characteristics that affect the productivity and should therefore be the inputs for a new model (Papáček, et al., 2004).

This can be achieved by using a kinetic model based on the Monod equation combined with Lambert-Beer's law, as proposed by (Hermanto, 2009). This will enable to specify for different reactor designs by accounting for differences in mixing, light path, culture density, light regime and algae light absorption coefficient and maximum specific growth rate.

4. Microalgae growth model

The main shortcomings of the previously described model from Jonker were that it was not specified for the type of reactor that is used and the orientation of that reactor. These are important parameters influencing the light intensity on the microalgae. The calculations for the microalgae growth were based on the application of growth reducing factors retrieved from literature, not on a kinetic model of the microalgae growth providing real insight in the factors that influence the productivity.

The new model that is used for this section of the analysis is based on the work of Slegers from Wageningen University. It consists of two parts: calculations on the light that falls onto the reactor and a model for microalgae growth based on the work of Geider (Geider, MacIntyre, & Kana, 1997). This model from Slegers (Slegers, Wijffels, Straten, & Boxtel, 2011) is specifically about flat panel reactors, but there are two master theses on tubular reactors and open ponds available (Beveren, 2011) and (Lösing, 2011) respectively). The model is simplified and adapted for the purpose of analysing the mass and energy balances of microalgae growth. The next section describes the model and adaptations made to it in more detail.

Light

The first part consists of five sub models representing the different factors that affect the light incident on microalgae (Slegers, Wijffels, Straten, & Boxtel, 2011):

- the light incident angle
- light input on the reactor (direct and diffuse light)
- the effect of neighbouring reactors
- transmission though the reactor wall
- the light gradient inside the algae culture

The first four are mainly affected by the reactor geometry and characteristics; the light gradient is also affected by characteristics of the algae culture since algae absorb the light and that influences the intensity of the light inside the reactor. All formulas in the next section are from (Slegers, Wijffels, Straten, & Boxtel, 2011)

For open ponds only the light incident angle, the transmission of light from air to algae culture and the light gradient inside the algae culture are important.

Light incident angle

The light incident angle is affected by day-night and seasonal changes, the location and the orientation of the reactor. The change in solar incident angle θ over time in case of an open pond equals the solar elevation angle α_v , determined by the location (longitude and latitude), the season and the time of the day.

The incident angle for direct light to the horizontal surface θ_z (°) can be calculated by:

$$\cos(\theta_z) = \cos(\varphi)\cos(\delta)\cos(\omega) + \sin(\varphi)\sin(\delta)$$
(7)

Where φ (°) is the latitude of the reactor location, δ (°) is the declination of the sun, i.e. the angle between the rays of the sun and the plane of the equator and ω (°) is the hour angle, the angular displacement west or east of the local meridian due to the earth's rotation on its axis. An illustration of these parameters is given in figure 8.

The solar declination $\delta,$ can easily be calculated by using the day of the year:

$$\delta = 23.45 \sin\left(\frac{360(284+N)}{365}\right) \tag{8}$$

Where N is the number of a day in a year; starting at the first of January. This angle varies from 23.45 - 23.45 throughout the year.

The solar hour angle ω is the displacement of the sun from the local meridian at a certain point in time. By converting the difference between the actual time and the solar time into degrees, this displacement at the reactor location can be calculated. Noon in this timescale is the point when the sun crosses the local meridian.

$$\omega = \frac{360}{24} * 12 - t_{\rm solar} \tag{9}$$

This solar hour angle changes from 180 to -180 through one day.

Solar time is calculated using the difference in longitude between the reactor location and the local meridian, and the deviations from the rotation of the earth, *e* (representing the difference in length of a sundial and a day through the year).

$$t_{solar} = t + \frac{4(\lambda - \kappa) + e}{60}$$
(10)

 $e = 229.2 (0.000075 + 0.001868 \cos(\zeta) - 0.032077 \sin(\zeta) - 0.014615 \cos(2\zeta) - 0.04089 \sin(2\zeta))$ (11)

$$\zeta = (N-1)\frac{360}{365} \tag{12}$$

In case of a vertical flat plate photobioreactor or a vertically stacked horizontal tubular reactor the slope (β) and the surface azimuth angle (γ_{back}) affect the solar incident angle. This is illustrated in figure 8.



Figure 7 - Solar incident angle during one day in on June 21st for the two locations used in this research



Figure 8 - Illustration of surface azimuth angle γ_{back} , solar elevation α_v , zenith angle θ_z , solar incidence angle θ , hour angle ω and slope of the reactor surface β (Slegers, 2011)

The solar incident angle is calculated as follows:

$\cos(\theta) = \\ \sin(\delta)\sin(\varphi)\cos(\beta) - \sin(\delta)\cos(\varphi)\sin(\beta)\cos(\gamma) + \cos(\delta)\cos(\varphi)\cos(\beta)\cos(\omega) + \\ \cos(\delta)\sin(\varphi)\sin(\beta)\cos(\gamma)\cos(\omega) + \cos(\delta)\sin(\beta)\sin(\gamma)\sin(\omega)$ (12)

In this case β , the slope of the reactor with respect to the ground surface, γ , the reactor azimuth angle (the angle between the normal of the reactor and south and φ), the latitude of the reactor location, are reactor characteristics and thus fixed values. This solar incident angle determines for a large share the amount of radiation that reaches the algae. This angle varies per hour and throughout the year, and also for reactor orientations and different locations. In figure 9 an example is shown of the variation in solar incident angle for different locations (on the northern hemisphere) for three different types of reactors: a horizontal surface such as an open pond, a 45° degrees inclined panel and a vertical standing wall. For example a reactor on the North Pole receives on the day that the sun is right above the equator, light that is parallel to the ground surface, so for a vertical reactor the angle with the normal of the reactor is 0° and for an open pond this angle is 90°.



Figure 9 - Light incident angle at March 21 for changing latitude

Light input on single and multiple reactors

Single panels

To calculate the light intensity real data from weather databases is used. To convert this radiation data into values for vertical or tilted panels geometric factors for direct irradiation at the front and the back are used as proposed by Slegers *et al.* (2011):

$$G_{direct,front}(t) = \frac{\cos(\theta)(\beta_{front},\gamma_{front})}{\cos(\theta_Z)}$$
(14)

$$G_{direct,back}(t) = \frac{\cos(\theta)(\beta_{back},\gamma_{back})}{\cos(\theta_z)}$$
(15)

Not only does the irradiation intensity, also the incident angle differs for the backside for the panel; the incident angle $(\cos(\theta) \beta_{back}, \gamma_{back})$ is calculated using the slope and azimuth angle of the backside:

$$\beta_{\text{back}} = 180 - \beta_{\text{front}}$$
(16)
$$\gamma_{\text{back}} = \gamma_{\text{front}} + 180$$
(17)

The factors for diffuse radiation are only influenced by the slope (β) of the front and back of the panel.

$$G_{diffuse,front}(t) = \frac{1 + \cos(\beta_{front})}{2}$$
(18)

$$G_{diffuse,back}(t) = \frac{1 + \cos(\hat{\beta}_{back})}{2}$$
(19)

The third factor is reflection of diffuse radiation by the ground surface. This is determined by the reflectivity ρ of the ground surface and the slope (front and back):

$$G_{reflect,front} = \rho \frac{1 + \cos(\beta_{front})}{2}$$

$$G_{reflect,back} = \rho \frac{1 + \cos(\beta_{back})}{2}$$

This result in three geometric factors (direct and diffuse radiation and reflection) that affect the total light input at the both sides of the photobioreactor. These are multiplied by data for diffuse and direct irradiation for both sides of the reactor according for formulas 20 and 21:

$$I_{o,front}(t) = (G_{direct,front}(t) + G_{reflect,front})I_{hor,direct}(t) + (G_{diffuse,front} + G_{reflect,front})I_{hor,diffuse}(t)$$

$$I_{o,back}(t) = (G_{direct,back}(t) + G_{reflect,back})I_{hor,direct}(t) + (G_{diffuse,back} + G_{reflect,back})I_{hor,diffuse}(t)$$
(20)
$$(20)$$

Multiple panels

In case of multiple parallel panels shading has to be taken into account. The shadow height is given by:

$$H_{shadow}(t) = \frac{h - \tau \tan(90 - \theta_z)}{\sin(\psi)}$$
(22)

 τ is the distance between the panels in metres, h is the reactor height, and the solar elevation is given by 90 – the zenith angle (θ_z). The angle between the solar rays and the panel (ψ) is given by $|\gamma_{front}| - |\omega|$ (the azimuth angle and the solar hour angle).

The diffuse light input also changes when panels are placed parallel. Closer to the ground surface the diffuse light intensity decreases depending on *u*:

$$u = tan^{-1} \left(\frac{y}{\tau}\right) \tag{23}$$

Where y is the height at which the intensity is calculated.

The diffuse light input can then be calculated by using the slope and this factor u for the diffuse light between parallel panels:

$$\begin{aligned} G_{diffuse,front}(t) &= 1 + \cos(\beta_{front} + u)/2 \end{aligned} \tag{24} \\ G_{diffuse,back}(t) &= 1 + \cos(\beta_{back} + u)/2 \end{aligned} \tag{25}$$

Combining diffuse and direct light input on parallel panels results in:

$$I_{o,front}(y,t) = G_{direct,front}(t)I_{hor,direct}(t) + G_{diffuse,front parallel}(y)I_{hor,diffuse}$$
(26)

$$I_{o,back}(y,t) = G_{direct,back}(t)I_{hor,direct}(t) + G_{diffuse,back \ parallel}(y)I_{hor,diffuse}$$
(27)

The light input on the reactor is based on data from the SoDa database. Hourly direct and diffuse irradiance on horizontal or tilted panes is used as an input for the model (Table 4). In case of analyses per hour for one day, and average is used of five days in order to diminish the influence of weather fluctuations. Figure 11 shows an example of the irradiance profile of direct irradiance on a horizontal surface for the two locations used in this research.

Table 4 - Irradiance data (SoDa, 2004)										
Location	Narbonne Rotterdam									
Inclination	0°	45°		90°		0°	45°		90°	
Month	Direct	Direct	Diffuse	Direct	Diffuse	Direct	Direct	Diffuse	Direct	Diffuse
1	74.7	100.3	40.0	98.2	32.8	29.3	38.1	20.6	42.0	16.4
2	111.1	125.2	50.5	108.3	38.8	54.0	53.1	33.8	51.9	25.0
3	160.0	143.0	65.0	102.7	45.3	96.4	70.0	54.5	56.1	37.3
4	216.0	153.8	74.2	77.8	45.8	187.3	143.2	74.4	89.8	48.3
5	264.4	163.6	79.5	58.2	43.9	232.0	145.4	85.6	67.9	50.8
6	305.7	184.9	79.2	53.4	40.6	274.2	172.7	86.2	70.1	48.7
7	297.9	186.6	78.1	59.5	41.2	218.8	128.2	83.3	56.1	48.4
8	234.6	161.5	70.9	69.6	41.7	170.8	113.9	69.7	63.0	43.5
9	192.9	166.5	68.3	106.6	46.3	140.0	124.1	60.9	91.6	42.9
10	104.3	88.9	53.3	71.6	37.6	80.1	88.7	41.6	80.9	31.8
11	78.3	99.0	40.1	94.0	32.1	29.8	31.5	20.6	33.4	15.5
12	64.9	96.8	35.3	98.2	29.9	19.2	21.9	14.6	25.0	11.1
Annual										
average	175.2	139.0	61.2	83.1	39.7	127.6	94.2	53.8	60.6	34.9



Figure 10 - Direct irradiance on a horizontal surface per hour

Transmission and reflection

Light falling on the reactor is partially reflected by the interface between the air and the reactor wall and by the interface between the reactor wall and the microalgae culture. This is influenced by the difference in refractive indices and the angle of incidence which equals the angle of solar elevation according to the Fresnel equations in (28) and (29).

$$R_{s} = \left[\frac{\eta_{i}\cos(\theta_{i}) - \eta_{t}\sqrt{1 - \left(\frac{\eta_{t}}{\eta_{i}} * \sin(\theta_{i})\right)^{2}}}{\eta_{i}\cos(\theta_{i}) + \eta_{t}\sqrt{1 - \left(\frac{\eta_{t}}{\eta_{i}} * \sin(\theta_{i})\right)^{2}}}\right]^{2}$$
(28)

$$R_{p} = \left[\frac{\eta_{i}\sqrt{1-\left(\frac{\eta_{t}}{\eta_{i}}*\sin(\theta_{i})\right)^{2}} - \eta_{t}\cos(\theta_{i})}{\eta_{i}\sqrt{1-\left(\frac{\eta_{t}}{\eta_{i}}*\sin(\theta_{i})\right)^{2}} + \eta_{t}\cos(\theta_{i})}\right]^{2}$$
(29)

Since normal sunlight is unpolarized, the overall reflection coefficient equals the average reflection coefficients for s- and p-polarized light.

$$R' = \frac{R_p + R_s}{2} \tag{30}$$

The total light incidence on the culture volume is the light falling on the outside of the reactor(I_0) reduced by the fraction that is reflected on the air-reactor wall interface, the fraction that is reflected on the reactor wall-culture volume interface and low transparency of the wall material (T_m). This is expressed in equation 31.

$$I_{i}(t) = I_{0}(t)(1 - R'_{1}R'_{2})T_{m}$$
(31)

In case of an open pond, only the interface air-culture volume is relevant. The reactor wall of a photobioreactor can be made of different materials. In this analysis plastic is considered as the reactor wall material. An overview of the data used for the calculation of transmission and reflection of light is provided in Table 5

Table 5 – Refractive indices (Slegers, Wijffels, Straten, & Boxtel, 2011)				
	symbol	value		
refractive index air	η_{air}	1.001		
refractive index reactor wall	$\eta_{plastic}$	1.570		
	η_{glass}	1.510		
refractive index culture volume	$\eta_{culture}$	1.330		
Transparency of the wall material	T _m	1		

Light gradient

A model for calculating the light gradient inside the reactor was developed by (Barbosa, et al., 2005) based on Lambert-Beer's law (equation 31). The light attenuation depends on the absorption coefficient (α), the concentration (C_x) and the light path (b).

$$I_{out} = I_{in} e^{\alpha * Cx * b}$$
(31)

The average light intensity (I_{ave}) inside the reactor was determined by integrating between x = 0 and $x = b_{reactor}$:

$$I_{out} = \frac{\int_{0}^{breactor} I_{in} * e^{-\alpha * Cx * x dx}}{\int_{0}^{breactor} 1 dx}$$
(32)

The solution to this integral is given by:

$$I_{\text{ave}} = I_{\text{in}} * \frac{1}{b_{\text{reactor}}} \left(1 - e^{-\alpha c * c x * b_{\text{reactor}}}\right) * \frac{1}{c_x \alpha_x}$$
(33)

This provides the ability of specifying for different light paths, algae concentration and absorption constant of the algae. This absorption constant is an algae specific parameter, determined by the characteristics of the chlorophyll in the algae. The light path is reactor specific, in case of a photobioreactor values between 3 and 10 centimetres are common, and in case of an open pond this can vary up to 50 centimetres. The light path has a direct influence on the photo concentration reaching the algae. This is depicted in figure 11. Algae concentration depends both on algae strain and reactor type, since the concentration is ideally as high as possible, but without preventing the light to penetrate all the way through the microalgae culture. Table 6 shows the parameters used in this part of the analysis.

Table 6 – Parameters for calculation of the light gradient in the reactor						
Reactor type	Light path (cm)	Absorption constant	Algae concentration	μmax		
Open pond	0.15	200 m2 /ltg	16 0/1	1 2		
Flat panel PBR	0.03	Nannochloropsis&	Nannochloropsis	1.5 Nannochloropsis		
Horizontal tubular PBR	0.05	Chlorella	1.5 g/l Chlorella	1.9 Chlorella		



Figure 11 - Light attenuation depending on the length of the light path of the reactor

Microalgae growth

The growth rate for microalgae in an open pond or photobioreactor depends on the light input, the maximum photosynthesis rate, the absorption coefficient, the chlorophyll-carbon ratio and the respiration rate of the algae. The function to describe this relation is based on an approximation of a PI-curve (productivity-light input) and described by (Jassby & Platt, 1976). The change in growth rate for increasing light intensity is shown in figure 12.

$$\mu = P_m^c \left[1 - \exp\left(\frac{-\alpha I_{pfd}\theta}{P_m^c}\right) \right) - r_m \tag{34}$$



Figure 12 - PI curve for Nannochloropsis (µmax =1.3)

The model considers photoacclimation and is valid for an ideally mixed system at constant optimal temperature. The growth rate μ depends on the irradiance experienced by the microalgae cell (I_{PFD}), and on the chlorophyll a-carbon ratio θ (g Chl/g C). This ratio can be adapted by the cell depending on the light intensity. This ratio is determined by a maximum θ_{max} ; the functional absorption cross-sectional area of the photosynthetic apparatus α (m²/mole photons) and the maximum carbon specific rate of photosynthesis r^c_m (s⁻¹). This rate in turn depends on the maximal specific growth rate and the rate of cellular maintenance r_m (h⁻¹).

$$\theta = \theta_{max} \frac{1}{1 + \frac{\theta_{max}\sigma I_{PFD}}{2P_m^c}}$$
(35)

Besides light input, nutrient concentration and temperature are factors that can limit algae growth. Since the nutrient concentration can be controlled easily in closed photobioreactors this is not considered as a limiting factor in this model, but temperature can be of influence in outdoor reactors. Blanchard suggests the following formula for the temperature effect (Blanchard, Guarini, Richard, Gros, & Mornet, 1996):

$$f_T = \left[\frac{T_{max} - T_w}{T_{max} - T_{opt}}\right]^\beta \exp\left[-\beta\left(\frac{T_{max} - T_w}{T_{max} - T_{opt}} - 1\right)\right]$$
(36)

Where T_{max} is the maximum temperature algae can still manage, above this temperature they will die, T_w is the water temperature, T_{opt} the optimum growth temperature for the algae and β a parameter that determines the sensitivity of the algae to temperature changes. Usually β is higher in summer than in winter months (Blanchard, Guarini, Richard, Gros, & Mornet, 1996). In fig X is the temperature factor is shown for different β 's by an optimum of 298 and a maximum of 313 K.



Figure 13 – Limiting effect of (suboptimal) temperature for different values of β

Since there is no clear data available for the value of β , this parameter is assumed to have a constant value, in order to add no complexity into the model without a solid basis. This value is set to 1.2, an average of the values used in (Blanchard, Guarini, Richard, Gros, & Mornet, 1996).

A model for open ponds should include a factor for changing water temperature during the day and year. This is described in (Lösing, 2011), this model however adds many parameters that all have very minor to insignificant effect on the overall results of the model and in that sense would make it too complex for this purpose. As an alternative, temperature changes are simulated by a goniometric function:

$$T_{seasonal} = T_{ampl} \sin\left(\frac{N}{365} * 2\pi - 0.5\pi\right) + T_{avg}$$

$$\tag{36}$$

N = day number T_{ampl} = temperature amplitude, T_{avg} = average annual temperature. For Rotterdam the average temperature used was 10°C with amplitude of 7°C, for Narbonne the average temperature used was 15°C with amplitude of 8°C. The annual change in local temperature is shown in figure 15. The same method was used for daily changes.



Figure 14 - Annual temperature change

Nutrient uptake

For the purpose of the environmental analysis in the next section nutrient consumption and oxygen production are calculated using stoichiometric factors. This is determined using the biochemical

composition of the microalgae biomass and the molar composition of the substrates and products (Pruvost, Vooren, Cogne, & Legrand, 2009; Grobbelaar, 2004)

$$CO_2$$
 + 0.148 HNO₃ + 0.014 H₂SO₄ + 0.012 H₃PO₄ + 0.751 H₂O →

$$CH_{1.715} O_{0.427} N_{0.148} S_{0.014} P_{0.012} + 1.437 O_2$$
(37)

These ratios are used to determine the amount of nutrients and carbon dioxide that are required (table 7).

Table 7 - Required amount of nutrient per unit of algae						
Name	Formula	Molecular weight	Ratio with algae			
		(g/mole)				
Algae	$CH_{1.715}O_{0.427}N_{0.148}S_{0.014}P_{0.012}$	23.499	1.00			
Carbon dioxide	CO ₂	44.009	1.87			
Oxygen	02	31.999	1.36			
Nitrate	HNO ₃	63.013	2.68			
Ammonia	NH ₃	17.031	0.72			
Urea	$CO(NH_2)_2$	60.056	2.56			
Phosphate	H_2PO_4	96.988	4.13			

This analysis is done for two species of microalgae: Chlorella sp. and Nannochloropsis. Chlorella is a green algae specie, single cell without flagella. It is well known since the 1940's, mainly for food supplementary potential at that time, but since then widely used for the production of biodiesel as well as other products. One of the main advantages of this species is that it is very suitable for growing a single strain in a large volume such as in an open pond, since it is well adapted to outdoor conditions (Mata, Martins, & Caetano, 2009). Nannochloropsis is a specie from the heterokont line that is mainly interesting for its high lipid content. It is well known as a marine species, but also occurs in fresh water. In 2012 the complete genome of Nannochloropsis gaditana has been sequenced (Radakovits, et al., 2012). For that reason it is often used for studies.

Table 8 – Symbols and values microalgae growth model						
Symbol	Name	Value, Unit				
θ	Solar incident angle					
δ	Solar declination					
ψ	Latitude of reactor	43.18°/51.93° (Narbonne/Rotterdam)				
β	Reactor slope					
γ	Azimuth angle					
ω	Solar hour angle					
Ν	Day number in a year					
t _{solar}	Solar time					
λ	Longitude of the reactor	3°/4.01° (Narbonne/Rotterdam)				
к	Meridian of reactor location	3°/4° (Narbonne/Rotterdam)				
e	Equation of time					
ζ	Day angle					
Io	Light intensity	µmole photons/m²/s				
Iout	Outgoing light intensity	µmole photons/m ² /s				
I _{in}	Incoming light intensity	µmole photons/m ² /s				
I _{ave}	Average light intensity	μmole photons/m ² /s				
Ipfd	Photosynthetically active light intensity	μ mole photons/m ² /s				
PAR	Photosynthetic active radiation	0.43 (Thimijan & Heins, 1983); (Janssen, Tramper,				
		Mur, & Wijffels, 2003				
a	Absorption coefficient algae	$200 \text{ m}^2/\text{kg}$ (Barbosa, Hoogakker, & Wijffels, 2003)				
L _x	Algae concentration	$0.5 - 1.6 \text{ Kg/m}^3$				
D(reactor)	Light path (reactor depth/diameter)	PBR)				
μ	Growth rate	S ⁻¹				
μ_{max}	Maximum growth rate	1.9/1.3 (Chlorella/Nannochloropsis) (Wagenen, et al., 2012)				
P ^c m	Maximum photosynthesis rate	S ⁻¹				
α^{clh}	Algae absorption coefficient	g carbon/g chl/m ² / μ mole photons				
Θ	Chlorofyll:carbon ratio	g chlorophyll a/g carbon (Slegers, Wijffels, Straten, & Boxtel, 2011)				
Θ _{max}	Max chlorofyll a and carbon ration in the cell	0.08 0.08 g chl a/g C (Slegers, Wijffels, Straten, & Boxtel, 2011)				
σ	Functional cross section of the photos.	$0.00001 \text{ m}^2/\text{mole photons}$ (Slegers Wiiffels				
	app. (m2/mole photons)	Straten, & Boxtel, 2011)				
r _m	Respiration rate	0.05 (Slegers, Wijffels, Straten, & Boxtel, 2011)				
Т	Temperature	K				
ft	Temperature factor	-				
T _{max}	Maximum temperature algae	313 K (Briassoulis, et al., 2010)				
Tont	Optimal temperature algae	218 K (Briassoulis, et al., 2010)				
Tw	Water temperature	К				
β	Temperature sensitivity parameter	1.2 (Blanchard, Guarini, Richard, Gros, & Mornet, 1996)				
Tampl	Temperature amplitude	7/8°K (Rotterdam/Narbonne)				
Tavg	Temperature average	283/288°K (Rotterdam/Narbonne)				
Tref	Reference temperature	293 К				

5. Results

This section shows the productivity of two microalgae strains in different growth conditions and for using different types of reactors according to the model that was developed in the previous section. The model especially enables for comparing the effect of one or two parameters over the course of a year, for a specific season, or during one day. The two species used in this analysis are Nannochloropsis sp. and Chlorella sp. Open ponds and two types of photobioreactor (flat plates and tubular reactors) are assessed. The photobioreactors can be placed at various inclinations, three options are assessed here: horizontal (0°), 45° inclined and vertical (90°). Two locations are assessed: the harbour area in Rotterdam (NI) and the harbour area in Narbonne (Fr). Each of these situations is put into the model and the results are compared based on volumetric and areal productivity. First this section will start with a description of the specific results per cultivation technology, followed by a general comparison of the overall results. A complete overview of the daily and annual volumetric and areal productivities for each technology is provided in table 11.

Open pond

For microalgae cultivation in open ponds it can be seen from the model that Chlorella species is more productive than Nannochloropsis. This is due to the fact that Chlorella has a higher maximum growth rate, although a slightly lower concentration was used according to optimum values found in literature. For both locations the annual change in productivity is shown in figure 15. The effect of the different algae species increases with lower latitude. It also shows clearly that the seasonal differences are larger for south France than for The Netherlands, despite the fact that the differences in irradiation are larger in higher latitude regions during the season.



Figure 15 – Annual productivity in open ponds of Nannochloropsis and Chlorella in Rotterdam and Narbonne.

For the most optimal case, Chlorella on the location in Narbonne, the relationship of the pond depth and the concentration is further assessed (figure 16). It can be observed that a shallow pond is more productive per unit of volume. The highest concentration of 1.5 g/l is the most productive for ponds with a depth less than 15 centimetres. For ponds deeper than 50 cm the lowest of the three assessed concentrations (0.5 g/l) is slightly more productive. This can be explained by the fact that a lower concentration allows for better light penetration. In general it can be stated that for shallower ponds (> 30 cm) microalgae concentration is a limiting factor; higher concentrations result in a higher productivity. This is then limited by the nutrient and carbon dioxide uptake and concentrations the algae can survive.



Figure 16 - Effect of concentration in relation to pond depth on the productivity (Chlorella, Narbonne)

When a closer look is taken at the daily changes in productivity in each season it can be seen that the differences in location are especially large in the winter months. In figure 17 an experiment with Nannochloropsis at 1.6 g/l in an open pond is shown for one day in each season. For the irradiance data an average of five days around the actual date was taken in order to avoid the influence of weather fluctuations.

The productivities are almost similar in the summer for both locations, only a shift in daylight time can be observed due to one degree difference in longitude, but the profiles are comparable. This is due to the fact that the algae in both locations receive sufficient light, and probably reach a maximum in productivity. In winter times however, the system in Rotterdam has very low production, both due to a short daylight period and a low peak at noon. Note that the irregularity in the production profile in Rotterdam is due to using (average) actual irradiance data. In case of a discontinue system that is only producing from spring to autumn, this factor of low production in northern regions becomes less important.



Figure 17 - Productivity of Nannochloropsis in an open pond during one day in four different seasons in Rotterdam and Narbonne.

Compared to literature the productivity of 0.38-0.55 gram per litre per day is relatively high but many different results are obtained. The aquatic species program reported 0.25-0.3 g/L/day for Chlorella, but in a 0.2 metre deep pond (Sheehan, Dunahay, Benemann, & Roessler, 1998). Other studies show for example 0.10 g/L/day in a 13 cm deep pond (Pushparaj, Pelosi, Tredici, Pinzani, & Materassi, 1997), but Brentner (2011) on the other hand uses a areal productivity of 48 g/m²/day that is similar to the outcomes of this research (37-55 g/m²/day), corresponding to a volumetric productivity of 0.24 g/L/day that is lower than in this research, but that is due to the difference in pond depth (Brentner, Eckelman, & Zimmerman, 2011).

In general, volumetric productivities according to this model are slightly over estimated, but for the purpose of comparison of different cultivation systems and conditions it is within acceptable range and similar to other modelling results.

Tubular reactors

When using a tubular reactor, Chlorella is the slightly more productive microalgae stain. A system of horizontally placed tubes is the most productive per unit of volume over the course of a whole year, especially in Narbonne. A horizontal system obviously makes more effective use of the available irradiation per litre of microalgae culture volume, but has a lower areal productivity since there is only one layer of tubes on the ground area, whereas a vertically stacked system has multiple (up to 15) tubes on top of each other although they are horizontally further spaced apart.

Table 9 – Annual productivity for tubular reactors (g/l year)					
Location	Inclination	Nannochloropsis	Chlorella		
Rotterdam	0°	301.2	364.1		
Narbonne	0°	377.8	478.9		
Rotterdam	45°	209.1	242.2		
Narbonne	45°	245.7	290.0		
Rotterdam	90°	105.4	116.7		
Narbonne	90°	118.1	131.4		

The annual productivity of a tubular photobioreactor system in The Netherland is lower than that of a system in South France, but in case of a vertically stacked system of horizontal tubes the difference becomes very little. This has to do with the angle of incidence of the incoming light; in higher latitudes the solar elevation angle is smaller, causing an advantage in the case of vertical standing panels. The downside is that a lot of light at the middle of the day in the summer months, when the solar elevation angle is the largest, is not used to an optimum.

Areal productivity differs from volumetric productivity for the three types of inclination, since it depends on the inclination angle how many reactors can be placed on an area. In case of a reactor of 1×1 m, only one horizontal reactor can be placed on a square metre, 1.4 reactors can be placed on one square metre in case of 45° degree inclination and 5 vertical panels (20 cm spaced apart). This affects the areal productivities of the various PBR's.



Figure 18 - Productivity for horizontally, inclined and a vertically stacked tubular system

The 45° inclined plane of stacked horizontal tubes with Nannochloropsis sp. is chosen for comparison of the seasonal differences and the differences between Narbonne and Rotterdam (figure 19). The variations become very small, except for the winter months. The productivity remains constant resulting in a daily productivity around 0.4 g/l/day for Nannochloropsis and 0.6-0.75 g/l/day for Chlorella.



Figure 19 - Productivity of Nannochloropsis in a tubular 45° inclined PBR

The results for Chlorella are very well comparable to other experiments, for example Chini Zitelli *et al* (1999) reports productivities of 0.73-0.83 g/l/day, or Molina Grima *et al.* (1994) showed results of 0.23 g/l/day, but with a microalgae concentration of 1.19 g/l (Molina Grima, et al., 1994; Chini Zittelli, Lavista, Bastianini, Rodolfi, & Vincenzini, 1999)

Flat plate reactors

The results for flat plate are very similar to the results from tubular reactors. Light is efficiently used due to short light paths and therefore high concentrations can be used. Chlorella is slightly more productive, especially in the inclined and horizontal system, for the vertical system this difference has become much smaller.

Table 10 – Annual productivity for flat plate reactors (g/l year)					
Location	Inclination	Nannochloropsis	Chlorella		
Rotterdam	0°	424.5	554.3		
Narbonne	0°	507.1	695.4		
Rotterdam	45°	317.7	476.3		
Narbonne	45°	362.5	374.3		
Rotterdam	90°	178.5	226.6		
Narbonne	90°	196.8	203.8		

Comparison of the changes during the year in productivity again show the largest difference in the summer in Narbonne, a more vertical standing panel allows for smoothening out thee peak during midday. Since vertical panels are the most common and also allow for higher areal productivity this data is used for the life cycle analysis.



Figure 20 - Productivity in flat plate PBR for various inclinations

The seasonal changes show little differences for location or for seasonal changes. Only the winter season has significantly lower results.

The results for vertical panels of 0.48-0.62 g/l/day are higher than what is reported in literature (0.2-0.3 g/l/day (Cheng-Wu, Zmora, Kopel, & Richmond, 2001), 0.36 g/l/day (Rodolfi, et al., 2009), but results for this reactor type varied more strongly with changing light path, concentration and different algae species.



Figure 21 - Productivity of Nannochloropsis in a 45° inclined flat plate PBR

General results

The annual productivity of Nannochloropsis sp. and Chlorella sp. in a horizontally placed cultivation system is significantly higher than in vertical photobioreactors. A closed photobioreactor has higher annual yields than an open pond. Important factors here are the slope of the reactor surface. A horizontal surface receives more irradiation than a vertical surface. A very clear increase in productivity can be observed during summer months; therefore this would be beneficial in the case of experiments that are only carried out during summer months. An inclined surface of 45° has a much lower peak in the summer but also diminishes the difference caused by latitude. In this situation a flat plate seems to be the most efficient system, for the three different degrees of inclination assessed in this research it is the system with the highest annual production. This has mainly to do with the short light path in comparison with a tubular system; the microalgae can make optimal use of the available light. Figure 22 shows an overview of the volumetric productivities for the various reactors, locations and algae species relevant for the life cycle analysis: the open pond, the horizontal and stacked tubular reactors and the vertical flat panels. According to the volume to area ratios in table 11, the areal productivities are calculated and depicted in figure 23. It can be observed that per square metre the flat plate reactors have the highest productivities, since they had higher productivity than the tubular reactors at the same slope, but the flat plates have a higher ratio of volume per unit area. A recent study of Draaisma et al. (2012) using the model from Slegers et al. (2011) on two types of micro algae in two locations showed comparable results on the differences between the cultivation systems. This study also assessed microalgae growth in open ponds, horizontal and stacked tubular reactors and flat panels, but for two different algae strains: Phaeodactylum tricornutum which is a little less productive than Nannochloropsis and Chlorella, but comparable, and Thalassiosira pseudonana, which is significantly less productive (Draaisma, et al., 2012). However, the profile of the productivities shows the same results as this research: for tubular reactors, the results correspond well; the flat panel reactors are more productive, although there is a difference due to different reaction conditions. This comparison also shows that the model overestimates for open ponds, these results are not comparable to the results of Draaisma et al. This difference cannot completely be explained by differences in productivity of the algae or different reaction conditions. It must be concluded that the model systematically overestimates the productivity for open ponds, probably due to the assumption of ideal reaction conditions such as sufficient CO₂ and nutrients, but that this is not true in experiments.



Figure 22 - Biomass productivity in gram per litre per day for each location and cultivation system



Figure 23 - Areal biomass productivity in gram per square metre per day for each location and cultivation system

Table 11 – Overview of productivity for different type of reactors						
Reactor type	Location	A/V ratio	Volumetric productivity		Areal productivity	
		m^2/m^3	kg/m ³ h	kg/m³ day	g/m² day	kg/m² year
Nannochloropsis						
Open pond	Rotterdam	10	0.016	0.378	37.8	13.8
Open pond	Narbonne	10	0.019	0.462	46.2	16.9
Horizontal tubular	Rotterdam	50.93	0.034	0.825	16.2	5.91
Horizontal tubular	Narbonne	50.93	0.043	1.035	20.3	7.42
Stacked tubular	Rotterdam	12.73	0.012	0.289	22.7	8.28
Stacked tubular	Narbonne	12.73	0.013	0.324	25.4	9.28
Vertical flat panel	Rotterdam	33.33	0.020	0.489	58.7	21.4
Vertical flat panel	Narbonne	33.33	0.022	0.539	64.7	23.6
Chlorella						
Open pond	Rotterdam	10	0.018	0.435	43.5	15.9
Open pond	Narbonne	10	0.023	0.547	54.7	20.0
Horizontal tubular	Rotterdam	50.93	0.042	0.998	19.6	7.15
Horizontal tubular	Narbonne	50.93	0.055	1.312	25.8	9.4
Stacked tubular	Rotterdam	12.73	0.013	0.320	25.1	9.16
Stacked tubular	Narbonne	12.73	0.015	0.360	28.3	10.3
Vertical flat panel	Rotterdam	33.33	0.026	0.621	74.5	27.2
Vertical flat panel	Narbonne	33.33	0.023	0.558	67.0	24.5



Figure 24 - Areal productivities of four algae strains, two from Draaisma *et al.* (2012), two from this research. North/East includes Rotterdam (this research) and Eastern Europe (used in Draaisma *et al.*), South includes Narbonne (this research) and Southern Europe (used in Draaisma *et al.*). The first two strains are less productive than the last two, but especially Phaeodactylum tricornutum should be comparable.

Conclusion and discussion

In general the closed photobioreactors show significantly higher productivities than open ponds per square metre. Volumetric productivities show smaller differences, but still a higher result, especially in horizontally placed reactors. Vertically placed reactors have lower volumetric productivities, but due to high area efficiency this type of reactor produces the largest amount of biomass per square metre.

This can be explained by a number of factors. The concentration of the microalgae has a positive effect on the productivity. Since all types of closed photobioreactors allow for good control of reaction conditions higher concentrations of microalgae can be used. Good mixing and degassing prevent the algae from competition for light, nutrients and carbon dioxide. The light path in photobioreactors is generally smaller (1-10 cm) than in open ponds (>10 cm). This allows for higher concentrations to be used as well, since the light can still penetrate to the inside of the reactor.

The model does allow for clear comparison of change in many parameters such as light, location, geometry of the reactor, algae strain and seasonal changes. The results for closed photobioreactors are in line with other models, but higher than other experimental data.

Modelling in excel does not allow for analysing the effect of multiple parameters in one run. This was solved by using a mathematical approximation for the temperature changes or irradiance data for example. This simplifies the results but also diminishes the effect of (irrelevant) changes in daily data due to for example weather conditions. Since multiple year average irradiance data was not available on an hourly basis, this approximations were a useful solution in this instance. For further development of the model, more sophisticated software should be used, to allow for easier analysis of the effects of multiple parameters.

6. Life cycle analysis of biodiesel production

Introduction

The second part of this research is an environmental analysis of three methods for microalgae cultivation using a downstream processing base case where the main product is biodiesel. For the environmental assessment the same three cultivation methods above described were considered: open ponds, flat plate photobioreactors and horizontal tubular photobioreactors. After the cultivation stage, multiple routes are possible to produce biodiesel, depending on the choices made on techniques used for harvesting, extraction and conversion into biodiesel. A base case of the most common and efficient techniques is chosen to analyse and compare the environmental effects of each cultivation technology.

Method

Life Cycle Assessment (LCA)

Life cycle analysis is a method to evaluate the environmental impacts associated with a products and processes. Energy, emissions and material flows are identified over the compete life cycle of a products and quantified to enable evaluations and comparison of different process options. This includes the use of raw materials, manufacturing, transportation, maintenance and disposal of materials. The process is formalized by the International Standards Organization (ISO, 1997).

The execution of a life cycle analysis has four phases as illustrated in figure 25:

• Goal and scope definition: sets the boundaries for the analysis, defines the level of detail and the functional unit.

• Inventory Analysis: quantifies emissions, energy and raw materials for each process and presents these in a process flow chart.

• Impact Assessment: quantifies and groups effects of the resource use and emissions into environmental impact categories such as emissions, land use, water and energy consumption or eutrophication.

• Interpretation: reports the results by allocating environmental effects to for example mass or economic value and evaluates the opportunities to reduce the environmental impact of the biodiesel production from microalgae (ISO, 1997; Guinée, et al., 2002).



Figure 25 - Framework of an LCA (Guinée, et al., 2002)

A life cycle analysis has a few limitations that have to be taken into account for this analysis. The two most important limitations for this research are: 1) the quality of the results depends on the quality and availability of the data; more accurate data will result in a more reliable result of the LCA, and 2) many assumptions must be made in relation to the definition of boundaries, the choice of data sources and the weighting and allocation of impacts. The effect of these limitations will be discussed in detail at the end of this section.

Aim and approach

The general approach of this LCA is to make an overview of all the streams for every step in the process, differentiated per cultivation technique, location and algae strain. The analysis will be based on CO₂

emission, water use, eutrophication, cumulative energy demand and direct land requirements. This overview of in- and outputs is compared to the production of petroleum diesel to put the results into perspective.

General system assumptions

To make a clear and realistic analysis of a relatively experimental process of the production of biodiesel from microalgae this section describes the assumptions made for this analysis.

First of all, out of many options in each stage of the production process a biorefinery route is chosen as a base case. For the cultivation of microalgae three technologies are considered: open ponds, flat plate photobioreactors and horizontal tubular photobioreactors. This has to do with two facts: flat plate and horizontal tubular reactors are already being used on a pilot scale and open ponds on a commercial scale, therefore data is available and these options are the most relevant for the actual project. Second is that the Climate KIC organization wants to install a pilot plant in two different locations: the Rotterdam harbour area and in South France near Narbonne. In the latter area open ponds are already used, for the Rotterdam location floating tubular plastic bags will be used.

The location in Rotterdam will be a 'slufter', an artificial brackish lake in the harbour containing sludge. Water containing salts will therefore be sufficiently available, nutrients and CO_2 need to be added to the reactors. Since this harbour is a large industrial area, CO_2 and nutrients can probably be retrieved from waste streams from nearby industries, in combination with the carbon capture and storage projects that are already developing in that area (Rotterdam Climate Initiative, 2012). The location in South France is not determined yet, but since Narbonne is located at the Mediterranean Sea, and has a harbour area as well, similar conditions might apply.

Harvesting of algae will be done by the sequential process of: centrifugation of slurry, high pressure homogenation, drying and particle size reduction. The next step is the extraction of the lipids by organic solvent extraction and then, the transesterification process takes place to produce biodiesel. Energy, equipment and chemicals needed for these processing steps would be required from external sources. Some waste streams such as water, organic solvent or acids can be recovered and re-cycled to the process, but of course, there will always be some losses and this implies that a small amount of fresh materials is required. Products other than biomass are a cake of cell material, the unused lipids and glycerol. The use of these outflows is not considered further, for this analysis.

Other important assumptions regarding the losses and efficiencies in each processing step are, for example, the efficiency of nutrient up-taking or the solvent losses. This is described in section 5.

Functional Unit

The functional unit for the first part of the analysis: the microalgae growth all material and energy requirements will be calculated per ton of biomass produced. For the second part; the production of biodiesel the analysis of land, water and CO_2 requirements as well as emissions will be one ton of biodiesel produced.

System boundaries

For the cultivation stage the system boundary is the cultivation system; the open pond or the photobioreactor, and it's direct in and outflows. The inflows are: purified flue gas, DPA, urea, micronutrients and fresh water the outflows: water losses, CO_2 and microalgae. This is depicted in figure 26.

The production of biodiesel from microalgal biomass is analysed according to the system boundaries shown in figure 27. Inflows are the microalgal biomass, extraction solvents, methanol and catalysts.

Outflows are water, extraction fluids, catalysts, un-transesterified lipids, glycerol, microalgae cake and biodiesel.

Another important boundary to consider is the level of detail. This analysis will be made based on the major environmental effects: CO_2 , water, eutrophication and direct land requirements. This will be done to a certain extent, in order to make the analysis unwantedly complex. For example, the energy requirements or CO_2 emissions for the production of certain materials, is not calculated but taken from a reference, without considering the exact production process.

In several stages of the process other greenhouse gasses than CO_2 are emitted, for example in the production of materials such as solvents. These are not taken into account since these emissions are secondary to the production of biodiesel and as a result the amounts are quite small. With regards to the material use of the production of biodiesel only the materials directly consumed in the process are analysed. Facilitating equipment such as pipelines or motoring equipment only have a low environmental impact compared to the direct impact of the microalgae production and are therefore not considered.



Figure 26 - System boundaries microalgae growth

Uncertainty and sensitivity analysis

Since large scale biodiesel production from microalgae is rather new and some steps in the process are still in a development phase several assumptions had to be made. This will create a degree of uncertainty in the results, for example the exact lipid content of the algae has a large influence on the results, but is rather unclear since different values appear in literature and this depends highly on the exact cultivation conditions. This kind of effects needs to be assessed by a sensitivity analysis in order to know the reliability of the results.

The following variables are expected to have a significant effect on the results and are therefore subject of a sensitivity analysis.

- Biomass productivity
- Lipid content
- Nutrients
- Biomass drying energy consumption
- Source of CO₂

The focus of this sensitivity analysis is the effect of these variables on the cumulative energy demand since that is the most important indicator of the performance of the production and refinery system.

Process description

The process of making biodiesel from microalgae involves a sequence of steps including harvesting of the algae, extraction of the oil and conversion into the product biodiesel. In each step chemicals, water and energy are required thereby affecting the environmental impact of the total process. For this analysis a base case of steps is selected, consisting of processes that are the most efficient or the most common and have good scale up potential (Halim, Danquah, & Webley, 2012). An overview of these steps according to

Halim *et al*. is provided in figure 27.

In order for microalgal biodiesel to be environmentally sustainable, the total CO_2 emitted in the downstream processing steps must be lower than or at least equal to the total CO_2 originally captured by the microalgal cells during cultivation. Therefore, processes selected in each step should aim at minimizing energy consumption (Halim, Danquah, & Webley, 2012).

Lipid composition

For the production of biodiesel fatty acids are the interesting component of microalgae biomass. A fatty acid molecule consists of a hydrophilic carboxylate group and a hydrophobic hydrocarbon chain. The characteristics used to designate fatty acids are the number of carbon atoms in the chain and the number double bonds (saturated of or unsaturated).

Fatty acids bonded to a head group form lipids. Depending on the nature of that head group fatty acids form a charged or an uncharged lipid. Examples of uncharged lipids are triacylglycerol; bonded to glycerol, or phospholipids. Uncharged lipids are usually used for energy storage purposes by microalgae





cells, charged lipids are for example used in the cell wall.

Algal oil also contains other types of neutral lipids such as hydrocarbons, sterols, ketones and pigments such as carotenes and chlorophylls. These lipids cannot be used for biodiesel production.

The lipid content and composition of the algae is affected by the growth conditions, such as nutrient and CO_2 supply, temperature, illumination intensity and phase of the microalgal life cycle. For example microalgae respond to nitrogen starvation by synthesizing more neutral (energy storage) lipids (Halim, Danquah, & Webley, 2012).

Harvesting

The first step is a dewatering process in order to harvest the microalgae cells from the dilute suspension that is in the open pond or the photobioreactor. Biomass can be harvested by gravity sedimentation,

centrifugation, filtration or flocculation to increase the microalgal biomass concentration to 10 up to 450 g dried microalgal biomass per litre of culture (Molina Grima, Belarbia, & Acién Fernández, 2003; Halim, Danquah, & Webley, 2012). A harvesting method is required to be able to process large volumes of biomass within a limited amount of time in order to be cost effective. Only for extremely low value products, such as biomass recovery from sewage-based processes gravity, sedimentation may be the method of choice. Centrifugal recovery, which is relatively energy intensive, is feasible for high-value products since it has the advantage of being able to process large volumes relatively rapidly. Another requirement when selecting a suitable harvesting method is the level of moisture that is left after harvesting. Sedimentation techniques generally leave more water in the product than centrifugation, possibly resulting in the necessity of a dehydration step afterwards that can increase the overall cost of the process (Halim, Danquah, & Webley, 2012).

For this LCA flocculation in combination with centrifugation and finally pressure filtration is used to increase the biomass concentration sufficiently for the extraction of the algal oils. Flocculation means the addition of a salt or other flocculation agent such as chitosan causes the negatively charged algae cells to aggregate and sediment quicker. Based on the result from section 5 the concentration in the open pond is 0.5% dried microalgae biomass per litre and 1.5-1.6% for closed photobioreactors. Flocculation is performed using natriumhydroxide. The concentration after flocculation is 2% dried microalgae biomass per litre. To increase the concentration to 16% the slurry is centrifuged and pressure filtration is used to bring the cell concentration to 40% dry weight per litre.

Pre-treatment

Before the lipids can be extracted from the cells the microalgae are pre-treated. This pre-treatment process increases the efficiency of the lipid extraction, by disrupting the cells and removing a large share of the water content. In this analysis high pressure homogenation at 1000 bar is used to disrupt the cell walls and to force the release of intracellular lipids to the surrounding medium, followed by an evaporation process using air at 60°C. The concentration after this pre-treatment has increased to 80% dry weight. This pre-treatment increases the efficiency, but it is an energy intensive step in the process of oil extraction (Halim, Danquah, & Webley, 2012).

Extraction

After the harvesting and pre-treatment, the microalgae can either be in the form of a disrupted concentrate or a dried powder. For this analysis it is an 80% dry weight disrupted concentrate that is exposed to an extracting solvent which extracts the lipids out of the microalgae cells. Since the pretreatment is already an energy intensive process, the lipid extraction needs a high level of specificity towards the desired products in order to reduce impact of further downstream purification steps. Ideally the extraction technology is selective to the acylglycerols that form the feedstock for biodiesel production and not to the polar lipid fractions and other neutral lipids such as free fatty acids, hydrocarbons, sterols, ketones, carotenes, and chlorophylls (Medina, Grima, Gimenez, & Ibanez, 1998). Several techniques are available for extraction of the lipids of which organic solvent extraction is one of the most common methods. A more green technology that is currently gaining more attention in research is supercritical fluid extraction, that does not require drying of the biomass and is therefore less energy intensive (Halim, Danquah, & Webley, 2012). In this analysis organic solvent extraction with a 2:1 volume ratio mixture of chloroform and methanol is considered. This step results in an aqueous phase, since the extraction is performed on a concentrate, that is purified by a filtration and a threefold distillation step to separate respectively the methanol, water and the glycerol, followed by evaporation to recover the catalyst used in the harvesting step from the water fraction. The organic phase containing the total lipid fraction is purified by using distillation and treated with urea to fractionate the lipids into acylglycerol fraction and non-acylglycerol lipids and fatty acids (Medina, Grima, Gimenez, & Ibanez, 1998). The methanol is recovered from this step after distillation (Halim, Danquah, & Webley, 2012).

Transesterification

For transesterification, lipids are reacted with an alcohol such as methanol, ethanol, isopropanol, and butanol. For this analysis methanol is used. This produces fatty acid methyl ester (FAME) or biodiesel. For this reaction a catalyst is requires, this can either be and acid or an alkali such as NaOH which is used in this instance. The reaction rates and conversions of alkali catalysts are much higher than of acid catalyst, they are already commercially used in the production of plant biodiesel (Huang, Chen, Wei, Zhang, & Chen, 2010)). Fatty acids undergo saponification when NaOH is added; therefore excess alkali catalyst must be added in order to compensate for this loss. The water content is a point to consider as well, since water reacts with free fatty acids in these alkaline conditions, consuming catalyst. If the water content is too high this will decrease the efficiency significantly (Chisty, 2007). After transesterification the mixture has to be purified to remove by-products (glycerol, NaOH, and excess methanol). This usually consists of two steps: fist the mixture is left to settle in order to separate the biodiesel/un-transesterified lipids phase from the glycerol phase, then the biodiesel phase is washed multiple times with water to eliminate any alkali catalyst and excess methanol (Chisty, 2007; Demirbas A., 2008).

Products

The products that are recovered from this process are the microalgae cake, omega 3 fatty acids, β carotene, biodiesel and glycerol. An analysis is made with three types of allocation: no allocation of the energy and material use and emissions, allocation according to mass of the products, and allocation to the economic value of the products. Table 12 shows an overview of the mass flow and economic value of the products that form the basis of this allocation.

Table 12 – Products distribution					
Products	Mass flow (kg/h)	Cost (€/kg)			
Microalgae cake	63.3	0.2			
Omaga-3	2.33	2.00			
β-carotene	1.40	2.00			
Astaxanthin	0.00	2.00			
Canthaxathin	0.00	2.00			
Zeaxanthin	0.00	2.00			
Fucoxanthin	0.00	2.00			
(Oil)	(28.71)	0.40			
Biodiesel	28.55	0.50			
Glycerol	3.00	0.20			

Results

20 cases were considered, based on the resulst of the productivities model; an open pond, a horizontal tubular reactor, a vertically stacked tubular reactor, horizontal flat plate and a vertical flat plate. These reactors are analyzed for two the two locations (Rotterdam and Narbonne) and for two microalgae species (Chlorella and Nannochloropsis). An overview is shown in table 13.

For every one of these 20 cases the requred area, volume and number of reactor units were calculated. Next the non renewable energy use and greenhousegas emissions were calculated for each case for every flow: DPA, urea, macronutrients, purified flue gas and the microalgae growth.

Table 13 – Non-renewable energy use and greenhouse gas emissions of the 20 cases studied ¹					
Reactor type	Location	Name	NREU	GHG	
			(MJ/kg microalgae)	(kg CO_2 eq/kg microalgae)	
Open pond	Rotterdam	Case 1	33.71	-0.052	
Open pond	Narbonne	Case 2	33.71	-0.052	
Horizontal tubular	Rotterdam	Case 3	21.92	-0.653	
Horizontal tubular	Narbonne	Case 4	21.92	-0.653	
Vertically stacked tubular	Rotterdam	Case 5	22.55	-0.621	
Vertically stacked tubular	Narbonne	Case 6	22.55	-0.621	
Horizontal flat panel	Rotterdam	Case 7	21.82	-0.658	
Horizontal flat panel	Narbonne	Case 8	21.82	-0.658	
Vertical flat panel	Rotterdam	Case 9	24.71	-0.514	
Vertical flat panel	Narbonne	Case 10	24.71	-0.514	
Open pond	Rotterdam	Case 11	34.78	0.005	
Open pond	Narbonne	Case 12	34.78	0.005	
Horizontal tubular	Rotterdam	Case 13	23.27	-0.582	
Horizontal tubular	Narbonne	Case 14	23.27	-0.582	
Vertically stacked tubular	Rotterdam	Case 15	23.58	-0.566	
Vertically stacked tubular	Narbonne	Case 16	23.58	-0.566	
Horizontal flat panel	Rotterdam	Case 17	25.05	-0.494	
Horizontal flat panel	Narbonne	Case 18	25.05	-0.494	
Vertical flat panel	Rotterdam	Case 19	26.05	-0.443	
Vertical flat panel	Narbonne	Case 20	26.05	-0.443	

¹) In cases 1-10 Nannochloropsis is used and in cases 11-20 Chlorella.

The most optimal case was case 3: a horizontal tubular photobioreactor, located in Rotterdam using Nannochloropsis. This had the lowest non-renewable energy use and (negative) greenhouse gas emissions during microalgae cultivation. An exeption was the horizontal flat panel, which had a slightly lower energy use and emission level, but is a configuration which is hardly used in practice, and was therefore not considered for the base case of the LCA.

The most optimal case, was used to analyse the energy use and greenhouse gas emissions of every step in the downstream processing. For the microalgae growht phase it was calculated that an area of 37037 m² is

Table 14 – Non-renewable energy use and greenhouse gas emissions for case 3 (no allocation)					
	NREU (MJ/kg microalgae) GHG (kg CO ₂ eq/kg microalgae)				
DPA	1.143	0.081			
Urea	8.210	0.414			
Purified Flue Gas	0.000	-1.830			
Micronutrients	0.260	0.002			
Microalgae Growth	12.306	0.680			
Total	21.919	-0.653			

needed to produce 100 kg microalgae and a culture volume of 2909 m³ equalling 6325 reactor units. Table 14 shows an overview of the energy use and emissions of the separate flows in microalgae cultivation.

The base case of downstream processes involved the horizontal tubular reactor, harvesting by flocculation with NaOH and centrifugation. Then pre-treatment by high pressure filtration followed by high pressure homogenation at 1000 bar and a drying step. The lipids are extracted by organic solvent extraction with chloroform/methanol (2:1 v/v). This results in two phases: an aqueous phase containing the methanol and the glycerol and an organic phase containing the lipids. These phases are both purified. The aqueous phase by high pressure filtration, distillation I (to recover the MeOH), Distillation II (to recover the water), and distillation III (to recover the glycerol); finally the catalyst is purificated by evaporation. The organic phase is purificated by distillation, fractionation with urea (3/1 Urea/PUFA (polyunsaturated fatty acids) Ratio; and evaporation. Finally transesterification is performed followed by a reaction with KOH, post transesterification purification means three separate distillation steps and neutralization.

Figure 28 and 29 show the non-renewable energy use and the greenhouse gas emissions of microalgae growth of the different flows. It can be observed that the urea, providing nitrogen to the algae, is a significant factor in the energy consumptions of the microalgae growth and in the greenhouse gas emissions as well. Using flue gas is a direct way of bringing the greenhouse gas emissions down or turn them negative.



Figure 28 - Non-renewable energy use of microalgae growth (MJ/kg microalgae, no allocation)



Figure 29 - Greenhouse gas emissions of microalgae cultivation (kg CO2-eq/kg microalgae, no allocation)

The results of the analysis of the energy use and greenhouse gas emissions during the steps in the downstream processing are shown in figure 30 and 31. The specific data can be found in Annex II. It can be observed that next to the urea use, the dewatering process and the purification of the organic and the aqueous phase after lipid extraction are the largest consuers of energy, due to the multiple destillation and evaporation steps involved. For greenhouse gas emissions the pattern is similar, but that the CO_2 inflow almost compensates for all emissions during the biodiesel production.



Figure 30 – Non-renewable energy use (MJ/kg biodiesel), for a horizontal tubular reactor, Rotterdam, Nannochloropsis



Figure 31 – Greenhouse gas emissions (kg CO₂ eq/kg biodiesel) for a horizontal tubular reactor, Rotterdam, Nannochloropsis

The overal values are hard to compare to literature since the large amount of parameters that can differ. However, Lardon (2009) for example shows the same ratios of energy use and energy production for a comparable process. 1 MJ of biodiesel equals an energy demand of 3.99 MJ and in this research the energy use of producing 1 kg of biodiesel (which equals 37.27 MJ) is 122.5 MJ; equalling 3.3 MJ energy use per MJ of biodiesel.

According to the data in Brentner *et al.* (2011) a similar process would use 4.9 MJ per MJ of biodiesel produced, which is higher than in this research, but comparable to Lardon's results. A difference between the three analyses is that Brentner uses a slightly less productive algae strain that is also lower in oil content (Scenedesmus dimorphus), as well as Lardon who uses Chlorella. An overview of the results from comparable research is shown in table 15.

Table 15 – Comparison of LCA studies on biodiesel production					
Name	MJ/MJ biodiesel	Description	source		
		Tubular reactor, flocculation,			
No allocation	3.29	drying, methanol extraction,	This research		
		esterification			
Mass allocation	0.95	и	This research		
Economic allocation	1.34	и	This research		
Best case	1.08	Flat plate, flocculation, supercritical methanol, anaerobic digestion	(Brentner, Eckelman, & Zimmerman, 2011)		
Base case 1	4.92	Flat plate, flocculation, drying, hexane extraction, esterification	(Brentner, Eckelman, & Zimmerman, 2011)		
Base case 2	7.82	Open pond, centrifugation, drying, hexane extraction, esterification	(Brentner, Eckelman, & Zimmerman, 2011)		
Normal N levels, wet extraction	3.99	Open pond, flocculation, centrifugation, hexane extraction, esterification	(Lardon, Hélias, Sialve, Steyer, & Bernard, 2009)		

Conclusions

The steps that are the most energy consuming are the steps that involve dewatering or distillation such as the harvesting and drying of the algae and the purification of the organic and aqueous phase after the organic solvent extraction. Exploring options that involve less drying such as using supercritical methanol in a wet combined extraction and transesterification or using self-flocculating algae could improve the environmental performance significantly.

For the microalgae growth the source of the nutrients has a direct effect on the overall energy use and emissions. Urea for example accounts for over one third of the energy use. Finding a different source for nutrients such as nitrogen, for example waste water from the sewage system or from fish cultivation industry, can hold potential to reduce the impacts of nutrients on the energy use and emissions of microalgae growth.

7. Conclusions

The purpose of this research was to explore the potential of cultivating microalgae for biodiesel production, by assessing different cultivation systems and the parameters that affect the microalgae growth. Microalgae technology is suitable for biodiesel production, but the specific impacts of the growth parameters and technologies used in different stages of the production of biodiesel are unclear. The research question to be answered was:

What are the expected environmental impacts of a microalgae biorefinery system for biodiesel production based on the analysis of different microalgae growth technologies?

To answer this research question first a model for microalgae growth was developed that accounts for all relevant variable and effects such as the type of photobioreactor that is used, the orientation, location and materials of this reactor, the specific growth characteristics of the microalgae and effect of temperature changes. The results of this model were used to perform a life cycle analysis on the production of biodiesel from microalgae.

The first model that was developed was very linear and straightforward and did therefore not take into account any feedback mechanisms in microalgae growth. It also did not include the reactor geometry thereby not allowing for comparison of the various systems. The second model allowed for comparison of the different systems based on changes in solar irradiation intensity (location, season, and reactor orientation), reactor geometry and materials, concentration and light utilization properties of the microalgae and temperature. This model performed well for closed photobioreactors compared to experimental data and other models. For open ponds the results were generally overestimated, probably due to the assumption of ideal reaction conditions. However, it was very suitable for comparing the effect of different reactor locations (Rotterdam and Narbonne) or different algae strains (Chlorella and Nannochloropsis). For further development of one integrated model for multiple cultivation systems, a module for the non-ideal conditions in open ponds could be added. Furthermore different software should be used to allow for easier assessment of the effect of multiple parameters.

The life cycle analysis showed that a horizontal tubular reactor with Nannochloropsis in Rotterdam is the least energy and emissions intensive one of the assessed configurations. A large share of the energy consumption and greenhouse gas emissions is caused by the nutrients provided to the algae, therefore finding a different source for these such as waste water, could be a way of reducing the impacts of microalgae cultivation.

The analysis of a base case for the production of biodiesel showed that the drying and purification processes are the most energy intensive steps. The total non-renewable energy use of the production of biodiesel from microalgae biomass is 3.29 times higher than the energy content of the biodiesel produced. When allocated to mass or economic value, considering the fact that biodiesel is not the only product that is extracted from the algae, the energy consumption is 0.95 or 1.34 MJ/MJ biodiesel produced respectively. This type of allocation shows that besides finding less energy intensive alternatives for the drying and purification it is also very important to consider co-production of multiple (high-value) products from microalgae in order to make the process more energy efficient and lower the emissions.

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Annex I

Irradiance data



http://solargis.info/doc/_pics/freemaps/1000px/ghi/SolarGIS-Solar-map-Europe-en.png

- Average annual irradiation Rotterdam: 1000-1100 kWh/m² = 114-125 W/m²
- Average annual irradiation Narbonne: 1500-1600 kWh/m² = 171-183 W/m²
- Compare: 127 W/m² in Rotterdam according to SoDa, 175 W/m² in Narbonne.

Annex II

LCA data

A. Non-renewable energy use of horizontal tubular reactor, Rotterdam, Nannochloropsis

	NREU (MJ/kg biodiesel)					
	NO	MASS	MASS	ECONOMIC	ECONOMIC	
	allocation,	allocation, NO	allocation,	allocation, NO	allocation, Credits	
	NO credits	credits	Credits (GLY)	credits	(GLY)	
DPA (2)	4.005	1.159	1.159	1.634	1.634	
Urea (3)	28.757	8.326	8.326	11.733	11.733	
Purified Flue						
Gas (1)	0.000	0.000	0.000	0.000	0.000	
Micronutrient						
S	0.091	0.026	0.026	0.037	0.037	
Flocculants	0.906	0.262	0.262	0.370	0.370	
Solvents	0.517	0.150	0.150	0.211	0.211	
Catalysts	0.042	0.012	0.012	0.017	0.017	
Fractionation						
agent	12.800	3.706	3.706	5.222	5.222	
Microalgae						
Growth	1.435	0.416	0.416	0.586	0.586	
Dewatering	16.132	4.671	4.671	6.582	6.582	
Cell						
disruption	7.663	2.219	2.219	3.127	3.127	
Drying	1.117	0.323	0.323	0.456	0.456	
Extraction	0.043	0.013	0.013	0.018	0.018	
Aqueous						
phase						
purification	32.637	9.450	9.450	13.316	13.316	
Organic Phase						
Purification	15.595	4.515	4.515	6.363	6.363	
Transesterific						
ation	0.771	0.223	0.223	0.314	0.314	
Credits (Gly)	0	0	-3.627	0	-3.627	
TOTAL	122.5	35.5	31.8	50.0	46.4	

	GHG (kg CO ₂ eq/kg biodiesel)					
	NO	MASS	MASS	ECONOMIC		ECONOMIC
	allocation,	allocation, NO	allocation,	allocation,	NO	allocation, Credits
	NO credits	credits	Credits (GLY)	credits		(GLY)
DPA (2)	0.284	0.082	0.082	0.116		0.116
Urea (3)	1.452	0.420	0.420	0.592		0.592
Purified Flue						
Gas (1)	-6.410	-1.856	-1.856	-2.615		-2.615
Micronutrient						
S	0.006	0.002	0.002	0.002		0.002
Flocculants	0.046	0.013	0.013	0.019		0.019
Solvents	0.033	0.010	0.010	0.014		0.014
Catalysts	0.002	0.001	0.001	0.001		0.001
Fractionation						
agent	0.646	0.187	0.187	0.264		0.264
Microalgae						
Growth	0.073	0.021	0.021	0.030		0.030
Dewatering	0.803	0.232	0.232	0.328		0.328
Drying	0.381	0.110	0.110	0.156		0.156
Extraction	0.056	0.016	0.016	0.023		0.023
Extraction	0.002	0.001	0.001	0.001		0.001
Aqueous						
phase						
purification	1.934	0.560	0.560	0.789		0.789
Organic Phase						
Purification	0.925	0.268	0.268	0.377		0.377
Transesterific						
ation	0.046	0.013	0.013	0.019		0.019
Credits (Gly)	0.000	0.000	-0.374	0.000		-0.374
	0.279	0.081	-0.293	0.114		-0.260

B. Greenhouse gas emissions of horizontal tubular reactor, Rotterdam, Nannochloropsis