ALMA MATER STUDIORUM
UNIVERSITA' DI BOLOGNA

SCUOLA DI SCIENZE
Corso di laurea magistrale in ANALISI E GESTIONE DELL’AMBIENTE

Life Cycle Assessment of biodiesel production from micro-algae: a case study in Denmark

Tesi di laurea in: Sistemi di gestione ambientale, di politica ed economia ambientale

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II Sessione
Anno Accademico 2012/2013

Summary

ABBREVIATIONS

AD anaerobic digestion

AP acidification potential

CED cumulative energy demand

CHP combined heat and power unit

EP eutrophication potential

FER fossil energy ratio

GWP Global warming potential

HDPE high density polyethylene

HT human toxicity

LCA Life Cycle Assessment

LCIA Life Cycle Impact Assessment

LDPE low density polyethylene

NER net energy ratio

ODP ozone depletion

POCP photochemical ozone creation potential

PO_4^{3-} phosphate

PBR photobioreactor

sCO_2 supercritical CO_2 extraction

ULS ultra-low sulfur

ABSTRACT

In recent years, biodiesel from algae has become an important research field, and several studies have been carried out on a laboratory- or pilot-scale in order to investigate biodiesel production from microalgae. Simultaneously, a number of Life Cycle Assessment (LCA) studies of biodiesel production from microalgae have been implemented; results are conflicting showing that only in some conditions this technology could be sustainable. Currently, algae have been cultivated in open ponds or in photobioreactors (PBRs): both technologies have advantages and disadvantages.

This study provides different scenarios on the eco-sustainability of the implementation of biodiesel production from microalgae cultivated in PBRs and located in Denmark on an industrial scale. LCA is the tool used to perform the assessment. The best available technologies for algal biodiesel production in PBRs were analyzed and compared. Each scenario has been compared also with diesel production performances. Finally, an evaluation of the parameters which most affect biodiesel production has been performed. The processes of algal biodiesel production taken into account are: cultivation, harvesting, the drying phase, oil extraction, transesterification, anaerobic digestion of residual biomass and the use of glycerol obtained from transesterification.

In the cultivation phase, both freshwater and wastewater have been alternatively considered; moreover, the use of both synthetic CO_2 and waste CO_2 have been alternatively assumed. In the harvesting phase, both flocculation with aluminum sulphate and lime, and centrifugation have been analyzed. Finally, in the oil extraction phase, both hexane extraction and sCO_2 (supercritical CO_2) extraction have been supposed. In these ways, 24 different and hypothetical scenarios are studied.

The basic scenario assumes cultivation in freshwater, use of synthetic CO_2, aluminum flocculation and hexane extraction, since these technologies are the most used for the production of other commercialized algal products such as cosmetic and pharmaceutical compounds.

The functional unit is 1 MJ of biodiesel. Secondary data were used and adapted in order to implement a biodiesel production system. IMPACT 2002+ is the LCIA method used. The main impact categories analyzed are: aquatic and terrestrial acidification (AP), carcinogens, aquatic

eutrophication (EP), global warming potential (GWP), non-carcinogens, non-renewable energy consumption, ozone layer depletion (ODP) and photochemical oxidant formation (POCP).

Since lipid content and extraction efficiency are relevant parameters in biodiesel production, sensitivity analysis is carried out on these parameters by varying the lipid content from 29% to 69% and the extraction efficiency from 0.91 to 0.95.

The main and relevant results are the following.

To develop microalgae biodiesel production on an industrial scale, considerable improvements must be achieved. In particular, different aspects of cultivation need to be enhanced, such as the use of wastewater and the insufflation of waste CO_2.

Harvesting (flocculation), drying and extraction processes (use of sCO_2) offer possibilities of improvement.

The valorization of co-products plays an important role in the biodiesel production process and in its development on a large scale.

An increase in the lipid content could significantly improve the environmental performances of biodiesel production. To date, the main limitation is that biomass productivity decreases when growing conditions increase the lipid content.

1 INTRODUCTION

1.1 THESIS GOAL

Biodiesel from microalgae is become an important research field. Recently, a few Life Cycle Assessment (LCA) have been investigating algal biodiesel production. In 2009, Lardon and coauthors performed a comparative LCA. Algal biodiesel was compared to fossil fuels and 1st generation biodiesel considering GWP; cumulative energy demand and others impact categories. In 2010, Stephenson and coauthors carried a LCA study comparing photobioreactors and open ponds, considering GWP and fossil energy ratio (FER). In 2011, Xu and coauthors worked on LCA about dry and wet route to produce biodiesel. These works were implemented on laboratory or pilot scale.

Therefore, it has been necessary to understand if algal biodiesel is really sustainable on industrial scale and which processes need to be improved and which of those are not sustainable.

Life Cycle Assessment (LCA) is a standardized methodology used in this work in order to evaluate the sustainability of the analyzed production processes. In fact, LCA allows assessment of environmental impacts and energetic loads of a system, considering all its life phases.

In recent years, a few LCA studies about biodiesel production from microalgae have been carried out on laboratory and pilot scale but not on industrial scale, yet.

This work aims to assess the sustainability of biodiesel production from microalgae in photobioreactors locating the production in Denmark.

Secondary data were adapted in order to develop a biodiesel production system on industrial scale.

The sustainability of biodiesel has been assessed choosing the best available technology and/ or process for algal biodiesel production.

A comparison between fossil fuels and algal biodiesel has been also carried out.

The final aim of this study is to provide a realistic scenario of how such technology could be implemented in Denmark.

This work of thesis is divided in four different parts: introduction, case study description, LCA of biodiesel production, conclusions and recommendations.

Chapter 1.2 describes features of fossil fuels, 1st and 2nd generation biofuels and microalgae. Then biodiesel production process is analyzed, investigating different technologies for each phase.

Chapter 1.3 provides an overall description of biodiesel production system and the technologies used.

Chapter 1.5 aims to describe LCA methodology and its four different phases. In this section, it is also performed a state of art of LCA on biodiesel production from microalgae.

Chapter 2 describes the case study in Denmark, highlighting each process and which technologies are used.

From Chapter 3 to chapter 7, the four LCA phases (goal and scope definition, life cycle inventory and modeling the system, LCIA and interpretation of results which contains comparison between different scenarios and sensitivity analysis) have been depicted.

Chapter 8 contains conclusions and recommendations.

References and appendix are last two parts of this work.

1.2 CURRENT CONTEXT

In recent years, the energy crisis has been taking an increasingly important role both economically and environmentally. Climate change, global warming and a possible depletion of fossil fuels (oil) are the main causes of this situation. By 2050 the oil reserves will be completely exhausted if the dependence on fossil fuels remains high (Markevicius et al., 2010). In fact, global primary energy demand is predicted to rise by 40% between 2007 and 2030 putting additional pressure on the fossil fuel dependent countries (Singh et al., 2010). Within the European transportation sector, the 98% of energy consumption is by fossil fuels (Kovacevic and Wesseler, 2010).

Figure 1.1 shows the increasing in oil use in last few years.

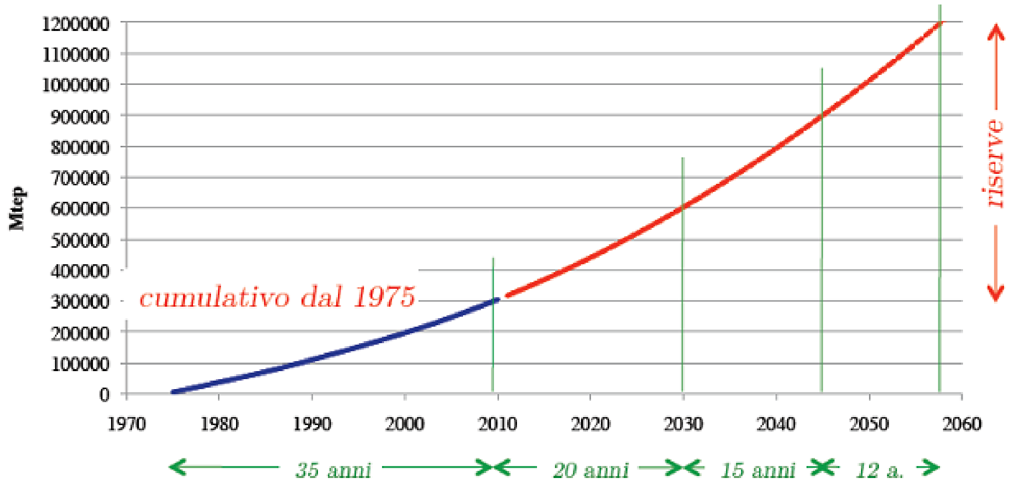


Figure 1.1: Increasing trend of use of oil. Within 2060, reserves of petroleum are used more than now (BP statistical review of world energy, 2011)

In order to avoid the exhaustion of fossil fuels, renewable energies can be an interesting alternative. Since fossil fuels contribute the most to emissions of greenhouse gases (GHG) in the atmosphere, it has been necessary to identify compatible mitigation strategies to minimize the excess of CO_2 emissions. Consequently, renewable and carbon neutral fuels are essential to both environmental and economic sustainability (Brennan and Owende, 2013).

Among renewable energies, biofuels are an attractive alternative to current petroleum based fuels. Biofuels refer to liquid, gas and solid fuels derived from biomass, also including dedicated energy crops and residue from agriculture. These biofuels are classified as first, second and third generation (Brennan and Owende, 2013). Due to their possible use in the transport sector and their similar features to fossil fuels, the fossil fuels dependence will be reduced and this will provide a number of environmental, economic and social benefits (Singh et al., 2012).

In EU strategy on biofuels (EU strategy for biofuels, 2006), the use of biofuels as a main candidate to replace fossil fuels is mentioned as follows:

- providing to decrease GHG emissions from transport;
- growing crops and using them in the country of origin;
- decreasing the dependency of oil imports;
- having similar oil properties;
- being blended with petrol or diesel (for example E85 gasoline in which 85% is ethanol and 15% gasoline).

Basing on European Directive, in 2008 the European Commission presented a directive (2009/28/CE) that aims to additionally promote the use of renewable energy sources and thereby contribute to climate change mitigation and a sustainable development. The directive establishes a target of a 20% share of renewable energy sources in energy consumption in which 10% is for the use of biofuels in transport by the year 2020 (Markevicius et al., 2010) as well as a reduction of 20% in GHG emissions.

Therefore, in recent years biofuels became an important research and development field for environmental sustainability.

1.2.1 Fossil fuels

The use of gasoline and diesel for road transportation will double in the next 25 years and GHG emissions will increase significantly (BP statistical review of world energy, 2011).

Road traffic already causes about 84% of all emissions from the transport sector in the EU. In EU, the share of traffic of total energy consumption is over 30% and it is constantly growing (Markevicius et al., 2010).

In order to compare diesel and biofuels, the main features of diesel are described: good cetane numbers (good ignition quality), cold-flow properties, low sulfur content, no aromatics, a good lubricity, oxidative stability, iodine value (useful for determination of the overall degree of saturation of the oil), density (not too high because there could be particulate emissions) and low viscosity. These main features of diesel are only a few important characteristics to assess its good quality. Cloud point and cold filter plug point are additionally considered (Ghasemi et al., 2012).

1.2.2 1st Generation biofuels

The first generation biofuels are based on food crops containing sugar and starch and vegetable oils. Oil crops are specifically used in the production of biodiesel while sugar in the grains is used to produce bioethanol (Demirbas, 2010)

The main crops used for biofuel are soy, rapeseed (Figure 1.2), palm, corn, wheat, sugar cane, sugar beet and sorghum. They are extracted using conventional techniques, which obtain biodiesel, biogas, bioethanol and syngas.

Figure 1.2: rapeseed cultivation

Some oil crops can be cultivated for energy but they have a low energy return i.e. the energy used for their production is the same or lower than the quantity of their combustion.

To produce energy, the cultivation of oil crops reduces land for agricultural purposes.

Instead of food, the growth of biofuel feedstock will require new cropping areas for food production. Since the actual feedstocks supply and domestic arable land available in Europe are not enough (Mata et al., 2013), competition with food prices in the market is one of the main consequences of land use change (LUC). The land use change can also be indirect (ILUC): forest and grasslands are replaced by oil crops cultivation. Losses in carbon pools and in biodiversity are the main environmental impacts (Markevicius et al., 2010) as well as a negative carbon balance in the atmosphere.

Moreover, there is competition with raw materials, feed, chemicals, fiber production and water. Due to intensive agriculture, there is a significant increase in the use of chemicals and fertilizers.

1.2.3 2nd Generation biofuels

The second generation biofuels from are produced by lignocellulosic biomass that are non-food. They are derived from cellulosic feedstock as waste wood, waste from plants, straw (in Figure 1.3), and grass but also from urban and organic waste i.e. waste from food, using thermochemical conversion processes (Brennan and Owende, 2013).

Figure 1.3: waste from straw are used to produce the 2nd generation biofuels.

A sugar extraction and fermentation with yeasts (*Saccharomyces ceversiae*) are performed to obtain bioethanol.

Anaerobic digestion is used to produce biogas (composed by CH_4 and CO_2) in anoxic environment in which microorganism like methanogens exist.

The 2nd generation biofuels are more efficient than those of the 1st one because they are generated by residual products and do not compete with food.

Since a high amount of cellulose is contained in the biomass, the investment and technology costs are high. In order to remove cellulose, pre treatments are necessary. This requires the use of chemicals, solvents and their recovery making it an expensive process (Singh et al, 2012).

1.2.4 3rd generation biofuels: Algae

The use of algae as energy source is not new. It was considered at the beginning of the 1950s but the idea was abandoned afterwards. In recent years, biodiesel from microalgae has not been produced on industrial and on commercial scale, yet.

At present, the multiple uses of algae have increased significantly and they are considered as the only alternative to current biofuel crops (Singh et al., 2010). For this reason, algae could possibly be utilized as a potential feedstock for biodiesel production.

Algae have several features that could allow sustainable energy production.

Algae do not compete with food and land since they grow in water (freshwater, seawater or wastewater).

Algae are already used for secondary and tertiary treatment of wastewater. Algae use nitrogen and phosphorus as nutrient removing them from water.

Algae cannot need chemicals, herbicides, pesticides in their growth (Kumar et al., 2010). As well as 2nd generation biofuels, algae establish atmospheric CO_2 for the growth of algal biomass. CO_2 emitted by algal biodiesel combustion is the equivalent amount to that used in the algal growth.

Algae growth rate is higher than those of terrestrial plants from which the 1st generation biofuels derive.

Algae can produce biodiesel as well as biogas from residual algal biomass (rich in carbohydrates) with anaerobic digestion and other valuable coproducts from proteins. In fact, microalgae are used for different valuable product in the current market. They can produce a wide variety of nutrients and secondary metabolite. Valuable co-products include carotenoids (β-carotene and astaxanthin) and long chain polyunsatured fatty acids (Hannon et al., 2010). In addition, carbohydrates and proteins fraction can be used for anaerobic digestion. This process can produce methane or animal feed in aquaculture industry (Hannon et al., 2010). Others minor commercial products from microalgae are extracts for cosmetics. Additionally microalgae can synthesize many molecules with commercial potential, such as toxins, vitamins, antibiotics, sterols, lectins and polyketides (Hannon et al., 2010).

Unfortunately, some problems affect the production of the 3rd generation biofuels. Until now, their commercial production has not been achieved on industrial scale in a cost efficient manner yet. Open ponds cultivation is affected by the maintenance of environmental condition in order to avoid thermal stress, bacteria contamination and variable sunlight (Kumar et al., 2010).

High costs for production facilities and a high energy demand are due to pumping and dewatering of biomass (Brennan and Owende, 2013). High energy is required to mix water and CO_2 in the open ponds with nutrients.

1.2.5 Features of algae used for biodiesel production

Microalgae are primitive plants without roots, stems and leave (thallophytes). They contain chlorophyll a as the primary photosynthetic pigment. Microalgae can be heterotrophic or autotrophic. Depending on their pigmentation, three main groups of algae are considered: green, red and diatoms.

One of the most important characteristics of algae is their high lipid accumulation, relating to biomass productivity.

The production of biodiesel is possible when algae have a higher lipid accumulation and this occurs only under certain conditions, for example under nitrogen stress conditions. Depending on algal strain, lipid content could reach more than 60% of algal biomass. Under these conditions, productivity of biomass decreases. This means an inverse relationship between biomass productivity and lipid content. High lipid content is generally coupled with a low productivity of biomass.

It is advised to use triglycerides (TAG) for biodiesel production in order to obtain such saturated fatty acids methyl esters (FAME) and glycerol as a byproduct (Figure 1.4). This chemical process is called transesterification and it is carried with methanol as a catalyst. Oxidation, free fatty acids and unsatured acids (up to 30%) could affect biodiesel production. The quality of biodiesel is good if the degree of unsaturation is low.

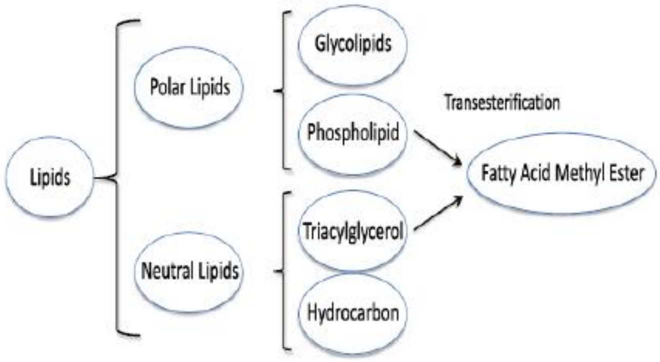


Figure 1.4: lipid used to produce a good quality biodiesel

Wastewater and lipid accumulation are directly related. In fact, wastewater is rich in nitrogen (N) and phosphorus (P) and this contributes to algae's rapid grow. Once that nitrogen is removed from the wastewater, a stress condition is reached and algae grow much less but the lipid accumulation is higher.

Rodolfi and coauthors (2009) carried out some experiments for the evaluation of algal species which are most appropriate for the production of biofuels. A study about the relationship of biomass productivity and lipid content was performed. The authors identified for the production of algal oil two species of freshwater algae *Chlorella* and *Scenedesmus*, and two of salt water, *Tetraselmis suecica* and *Nannochloropsis (*Figure 1.5).

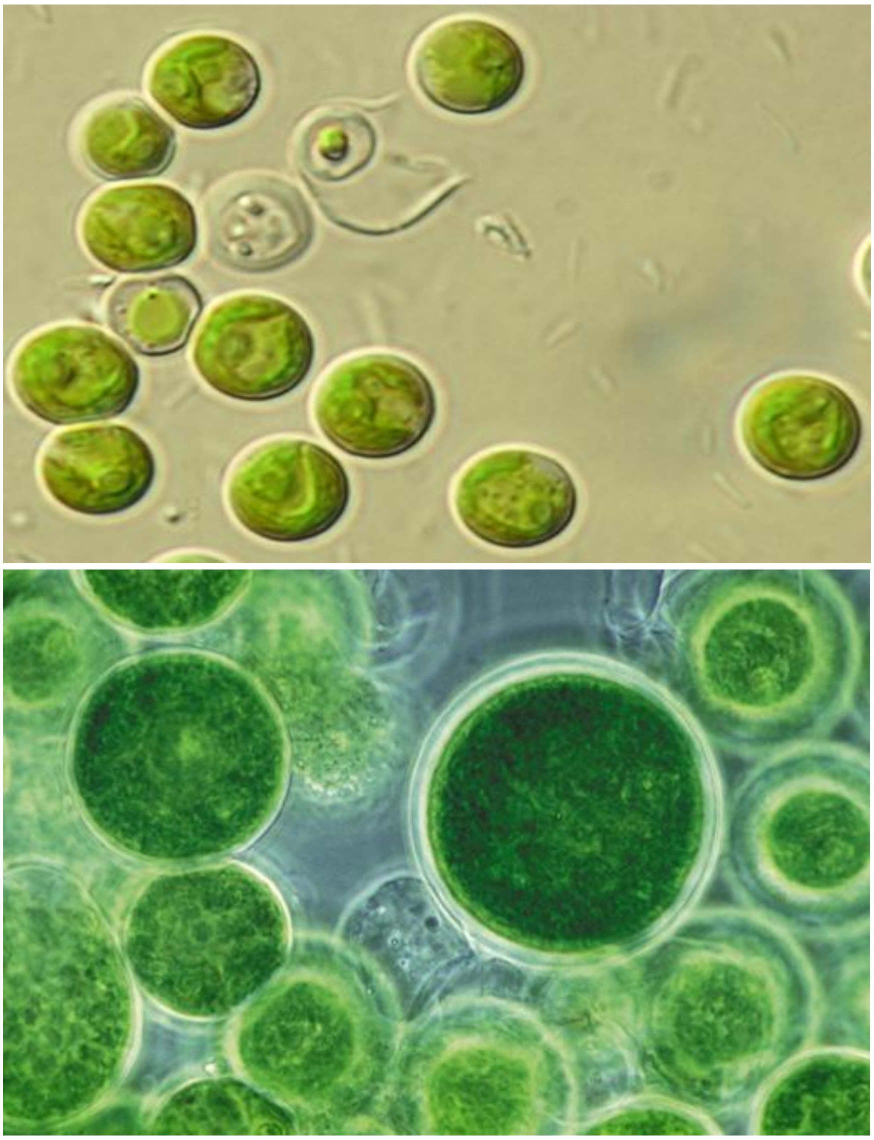


Figure 1.5: Chlorella vulgaris and Nannochloropsis

Selection of an appropriate algal strain should be based on the following characteristics:

- high lipid productivity and high lipid content in triglycerides;
- rapid growth rate;
- resistance to changes in environmental conditions (large range of temperatures for seasonal variations), and sources of contamination as bacteria;
- capability to grow in wastewater;
- high CO_2 fixation capability;

- high productivity of valuable co-products.

As stated by Brennan and Owende (2013), the ideal strain for biofuel production is shown in Figure 1.6.

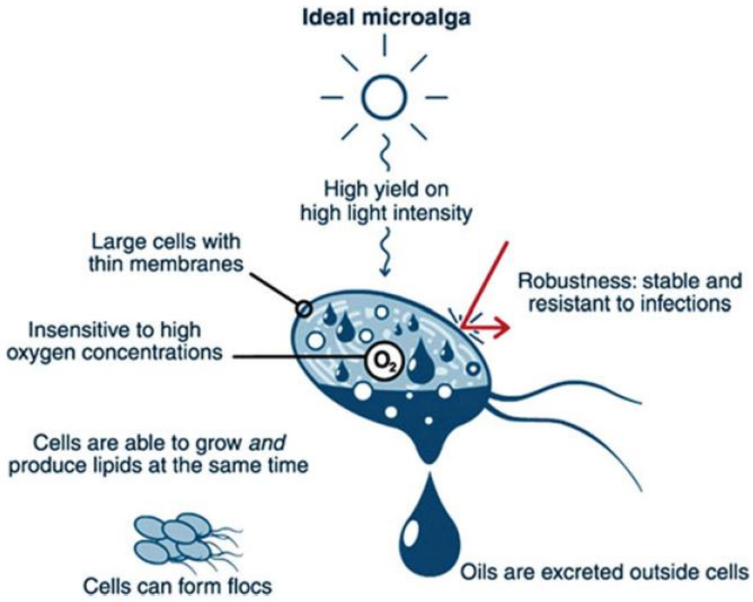


Figure 1.6:characteristics of an ideal algal strain for biodiesel production (Brennan and Owende, 2013)

1.2.6 Growth conditions

Several factors affect the growth of algae: nutrients (availability of N and P), sunlight and water type. In addition, the temperature, the pH and the dissolved oxygen (OD) can be considered as well. Growth rate and the lipid content depend on all of these factors.

Algae can grow in fresh water, salt water or wastewater. Wastewater is an interesting solution because it contains high amounts of organic carbon (estimated by the BOD - Biological Oxygen Demand), nitrogen and phosphorus used by algae for algal growth, as was already stated in section 1.2.5. Nutrients are limiting factors of algal growth because they affect the productivity of biomass. A high quantity of nutrients reduces the production of lipids (Kumar et al., 2010).

Sunlight is the main energy source for the algal growth, at the same time it is also a limiting factor. Given that, photosynthesis is highest above the saturation point; light excess causes photo inhibition to block the growth process. Water should not be too deep so that it ensures better sunlight. This is why photobioreactor, with its transparent surface, is the most efficient system for light exposure.

The temperature range for the algal growth varies from specie to specie. The optimum temperature is between 15-26°C. It should be noted that high temperatures with algal cultivation in outdoor tanks cause an increase in the rate of evaporation; therefore, the amount of water used in the stage of cultivation should significantly be higher.

Another important parameter is pH. Most of the algal species prefer a neutral pH (7.5-8). The amount of dissolved oxygen is important: if it is higher than 35 mg /l, there will be an inhibition of the growth process.

1.3 THE PRODUCTION PROCESS OF BIOFUEL FROM ALGAE

The production of biofuel is based on a process with different phases. Growth of algal biomass, harvesting, dewatering, algal oil extraction and transesterification are the most relevant phase. Different technologies can be used to carry out the biofuel production.

1.3.1 Cultivation

As algae grow in water, open ponds or photobioreactors can be used. Open ponds are the oldest and simplest systems for mass cultivation of microalgae. They are shallow ponds in which algae are cultivated.The pond is designed in a raceway configuration, in which a paddlewheel circulates and mixes the algal cells and nutrients (Demirbas, 2010). On the other hand, Photobioreactors are different types of tanks or closed systems in which algae are cultivated. Photobioreactors offer a closed culture environment, which is protected from direct fallout, relatively safe from invading micro-organisms (Demirbas, 2010).

1.3.1.1 Raceway Open Ponds

The open ponds can be built into the ground or in cement. The use of impermeable materials prevents water leakage. Open ponds are composed by circular channels in which water and algae in suspension are mixed with nutrients and gaseous $CO_{2.}$ To facilitate their mixture, a paddle wheel is used to increase contact between algae and nutrients. For the purpose of maximizing a gas exchange (Figure 1.7).

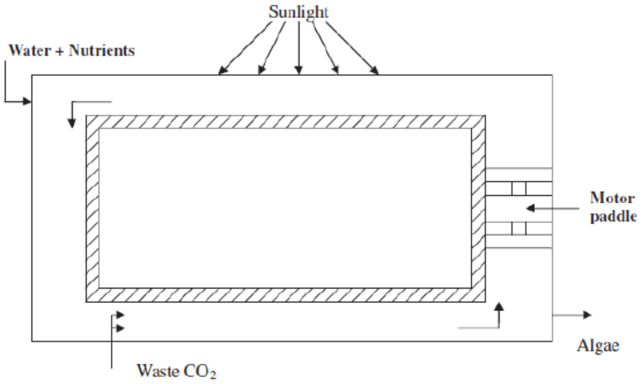


Figure 1.7:open pond system (Demirbas, 2010).

The water in these ponds is shallow enough for the algae to take sufficient sunlight. Few algal strains can grow in open ponds: *Spirulina*, *Dunaliella*, *Chlorella* and *Haematococcus*. They can tolerate stressful environmental conditions (Menetrez, 2012). In fact, possible contamination of different algal strains, pathogens or competing microorganisms can happen in open ponds. In this system, there is a lack of control due to evaporative losses, poor diffusion of atmospheric CO_2, high losses in water and CO_2, environmental fluctuations of temperature, pH and light. All these factors depend on poor or favorable weather conditions.

The production of open ponds does not necessarily compete with the land used for existing crop cultivation because algae can use wastewater or be built in areas with marginal crop production. The open ponds have low construction, maintenance and cleaning costs. However, they are not very efficient. This means that the productivity of algal biomass is low (Demirbas, 2010). On the other hand, algal harvesting and mixing processes have high energy costs (Figure 1.8).

Figure 1.8: Raceway open ponds

1.3.1.2 Photobioreactors (PBR)

Photobioreactors (Figure 1.9) are closed systems. They can be flat plates or tubular reactors, made in plastic or in glass, where algae cultivation is in suspension and CO_2 capture is efficient. Due to their high transparency, a higher amount of light is absorbed by algae and the biomass productivity increases in comparison to open ponds. For this reason, photobioreactor efficiency is higher than open ponds and the harvest time is shorter.

Since photobioreactors are closed system, contamination is not possible. In addition, less land surface is used in contrast to open ponds while increasing the control over growth conditions, especially temperature, light, pH, CO_2 and water.

On the other hand, photobioreactors have high capital, construction, operation costs whereas open ponds do not. In addition, a large amount of energy is necessary for the mixing of the water, nutrients and algae (Demirbas, 2010).

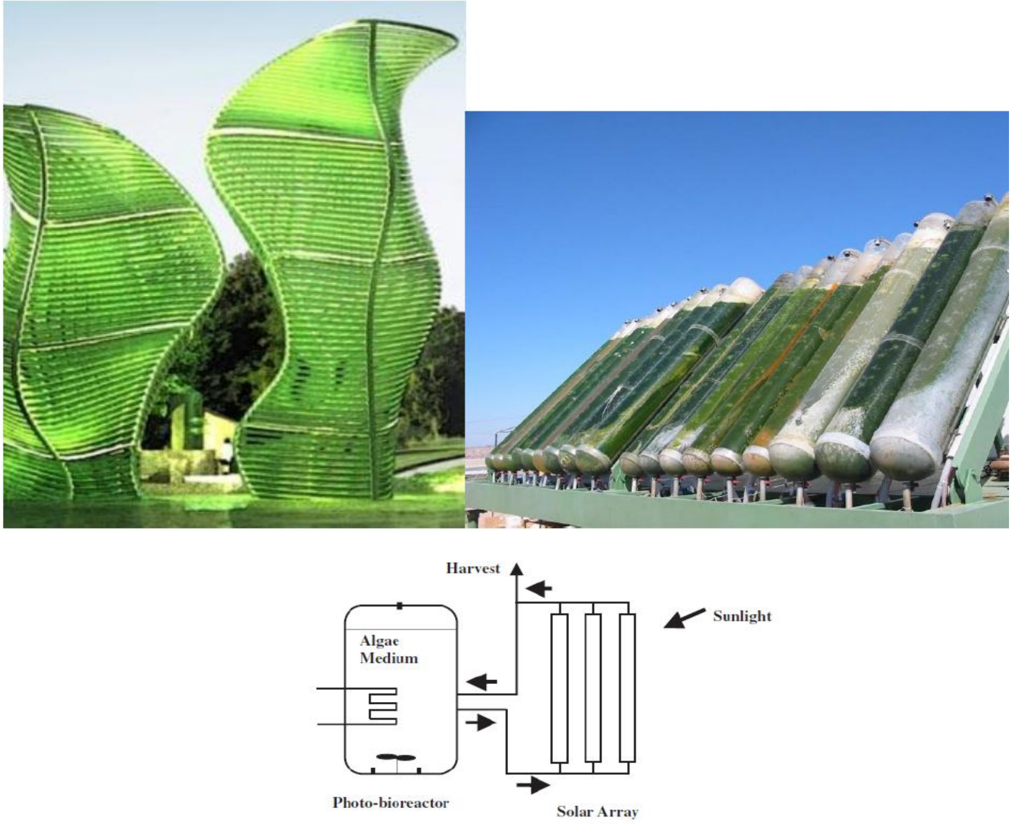


Figure 1.9: Photobioreactors (Demirbas, 2010)

The combined use of both these systems increases the productivity in a more efficient way. The first step is a fast cultivation of biomass in the photobioreactor. This allows maximum productivity of the biomass growth.

The second step is stressing cultivation in open ponds, in which the concentration of nitrogen is low (Singh et al., 2010) and high quantity of algal oil is produced (Ghasemi et al., 2012). Coupling open ponds and photobioreactors could be a cost-effective choice for cultivation of algae (Ghasemi et al., 2012).

1.3.2 Algal harvesting

Harvesting consists in the separation of algae from the water through complex and costly processes in terms of energy. The harvesting energetic costs often contribute to 20-30% of the total biofuel energetic costs (Singh et al., 2012). This process is divided in three steps: algal biomass recovery, dewatering and drying. Different problems have to be considered: low concentration of biomass, the small size of algal cells and the density of algal suspension which is similar to water density (Kumar et al., 2010).

Harvesting techniques may be different. They are made by chemical, physical or biological ways depending on the algal strain, cell density and cultural conditions (Demirbas, 2010).

The most used methods are the flocculation and centrifugation, followed by filtration or sedimentation (Pittman et al., 2011).

1.3.2.1 Flocculation

Flocculation is the first step and it is used to aggregate algal cells and to increase their actual size.

The surface of algal cells is negatively charged to prevent a suspended aggregation. When flocculants are added, they reduce or eliminate negative charges, thus, favoring cell aggregation. Afterwards, wet algal biomass is dried. Then, lipids and algal oil are extracted from it using CH_2Cl_2 (Pittman et al., 2011).

An alternative way to achieve this is through autoflocculation. Many species of unicellular algae spontaneously aggregate and precipitate to the bottom of the tank when the CO_2 stream is interrupted or when they are under environmental stress (Pittman et al., 2011).

In addition to chemical flocculants and autoflocculation, there are also natural flocculants. Among these, the seeds of *Moringa oleifera* are considered one of the best known natural flocculants. They are widely used in the treatment and purification of wastewater. These flocculants react by capturing the suspended particles. This process occurs due to the presence of protein in the seeds.

Dissolved air flotation (DAF) is often coupled with flocculation (Singh et al., 2010).

1.3.2.2 Sedimentation, centrifugation, filtration and other methods

Due to the low amount of biomass produced, sedimentation is a very common procedure followed by flocculation in the wastewater. Operation costs are low but the microalgae cells are not suitable for this process. Since algal cells have a small size, the harvesting rate is low (Pittman et al., 2011).

The centrifugation with rotating walls is a commonly used process, since it is fast and apt for different types of algae. Generally, this process is very efficient but expensive in terms of energy as well as capital and operation costs. As stated by Lardon and coauthors (2009) and by Singh and coauthors (2012), centrifugation is usually efficient but it is a very intense energy process.

Other methods used are mechanical filtration through filter presses or dry rollers which obtain a fraction of dry biomass. These methods are more expensive compared to the others (Singh et al., 2010).

1.3.3 Biofuels production

Different kinds of methodologies could be performed to obtain biofuel.

Oil extraction from dry biomass with solvent is one of the most relevant methods used.

Algal oil separation containing lipids from the remaining biomass is made by solvent extraction such as cyclohexane or hexane.

Afterwards, the oil and solvent are separated using a distillation process. This step is able to extract more than 95% of the total oil present in algae (Singh et al., 2010).

Another recent experimental method is supercritical CO_2 extraction. It is used in order to avoid dry algal biomass and the use of solvent. The extraction of oil occurs with wet algal biomass. Hexane can be replaced by CO_2 under supercritical conditions (100°C and 300 bar) as described by Brentner and coauthors (2011).

Another technology for extraction and transesterification is based on supercritical methanol, in which the drying phase is not performed as well as the previous process mentioned. Algal oil is extracted by wet biomass. Under supercritical conditions (240°-260°C and 82.7 bar), the combined use of methanol and water as chemicals replace the use of hexane (Patil et al., 2011). A high temperature and pressure are however limitations to this process.

A fraction of algal oil containing triglycerides is used to produce biodiesel as described in following section (section 1.3.3.1). The fraction of biomass containing proteins or carbohydrates can be used for anaerobic digestion or to produce bioethanol.

1.3.3.1 Transesterification

Algal oil is converted to biodiesel by transesterification. This is a chemical reaction between an ester (triglycerides) and alcohol which also uses a catalyst, as shown in Figure 1.10. Transesterification is a multiple step reaction where triglycerides are converted to monoglycerides. Afterwards monoglycerides are converted to esters (biodiesel) and glycerol as a by-product (Singh et al., 2012).

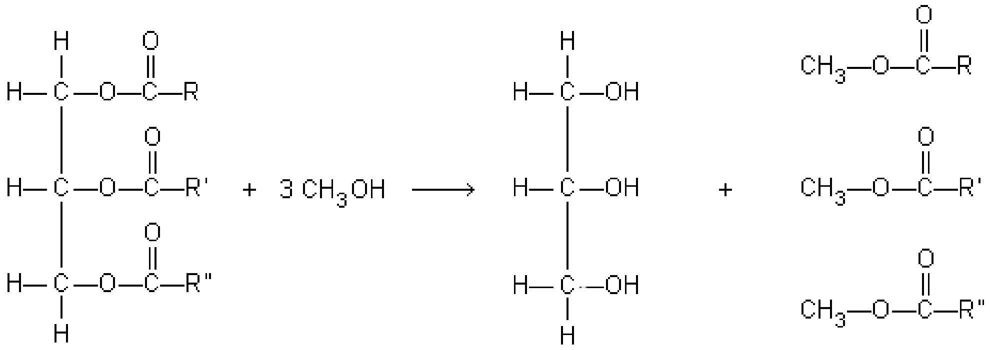


Figure 1.10: Transesterification : glycerol and methyester of fatty acid are the main products.

1.3.3.1.1 Glycerol

Glycerol is a by-product of transesterification during the biodiesel production.

Considering a conversion efficiency of 90% to produce biodiesel, the remaining 10% is glycerol, which is a chemical with a considerable high commercial value.

In the past, glycerol used to be produced by epichlorohydrin, a derivative of propylene. In the last few years, a minimal use of fossil fuels has been replaced by that of biodiesel. For this reason, the price of glycerol has decreased. A fall in the price of glycerol has lead to increased attention in the use of glycerol as a building block chemical. The increased production of biodiesel (and glycerol as a consequence) has creating a significant glut in the glycerol market, as stated by Johnson and Taconi (2007). A solution to this problem could be using glycerol to produce different chemicals with higher added value. This production leads to the development of glycerol biorafinery.

In view of its market capacity, the most important utilization of glycerol is for the production of glycol propylene. Glycerol is converted into glycol propylene by a chemical reaction called hydrogenation, thus replacing the use of propylene liquid made by natural gas. Interestingly, fossil fuels and natural gas are avoided in the production of glycol propylene when glycerol is used. Furthermore, glycol propylene replaces the production of glycol ethylene (toxic compound) at the same market price (Johnson and Taconi, 2007). To increase the value of by-products is relevant for the sustainability of biodiesel production.

1.3.3.2 Biochemical processes

The biochemical pathway interesting the fraction of biomass containing proteins or carbohydrates can lead to the production of bioethanol or biogas.

Bioethanol is produced in two phases namely the process of saccharification and fermentation. At first, it is necessary to break up the algal cell walls for starch extraction. During the fermentation phase, sucrose enzymatic hydrolysis occurs. The sugar is converted into ethanol through fermentation by yeast as *Saccharomyces cerevisiae* (Demirbas, 2010). Due to their high starch content, some microalgae species such as *Chlorella vulgaris* are a good feedstock for ethanol production (Brennan and Owende, 2013).

Biogas is produced by anaerobic digestion of residual algal biomass which derives from oil extraction. Anaerobic digestion is a bacterial conversion of residual biomass lacking oxygen over a temperature range of 30°-65°C. Biogas is the main product. Its composition varies generally from 55% to 71% for CH_4 and for CO_2 from 2.5% to 11.5%. There are some traces of NH_3, sulphide and hydrogen (Sialve et al., 2009). Biogas is used for the production of electricity or heat in a cogeneration plant.

To improve anaerobic digestion, other substrates are introduced to support it, such as waste paper, which improves the quality of biogas (Sialve et al., 2009).

1.3.3.2.1 Factors influencing anaerobic digestion

The amount of proteins contained in the algal cell walls can affect the process of anaerobic digestion. In fact an excessive amount of proteins causes the release of nitrogen in the form of NH_3 during the substrate digestion. NH_3 inhibits the anaerobic digestion process (Sialve et al., 2009).

If the concentration of some ions such as Na^+, Ca^{2+}, Mg^{2+} is too high, the release of NH_3 will decrease. To avoid inhibition and toxicity, it is necessary to operate with algae containing a low amount of protein in the cell walls. Another important factor in anaerobic digestion is the retention time: if it is high, the substrate will be degraded more efficiently by anaerobic bacteria to reach a more efficient conversion from biomass to biogas (Sialve et al., 2009).

1.3.3.3 Thermochemical processes

Thermochemical are used to convert wet algal biomass into different final fuel products, without drying. A thermochemical process converts the organic matter into a synthesis gas, by means of a partial oxidation in air, oxygen and / or steam at high temperature, typically in the range of 800-900 °C (Brennan and Owende, 2013).

Among the thermochemical processes, gasification, liquefaction and pyrolysis can be listed. In gasification with supercritical water conditions such as pressure of 220 bar and temperature of 600°C (Xu et al., 2011), the water changes its properties significantly acquiring a strong capacity to crush organic molecules creating syngas which is a mixture of H_2, CO_2 and CH_4 in a gaseous form. H_2 can be used for the subsequent hydrotreating process, replacing an external source.

Another technology used is liquefaction. It is carried out in low temperatures (300°-350°C) and with a high pressure. The main products are bio-oil, gas and reaction residues (Brennan and Owende, 2013). Liquefaction reaction is lead in an aqueous solution ("wet matter"), without requiring the drying of raw materials (Demirbas, 2010). The extraction of algal oil can also be done by the use of dichloromethane (CH_2Cl_2). Even if liquefaction may seem attractive for commercial exploitation, process reactors and systems for thermochemical liquefaction are in fact more complex and expensive (Brennan and Owende, 2013).

Pyrolysis is the decomposition of biomass to bio-oil, syngas and charcoal in a range of temperatures between 350°C and 750°C under oxygen deficiency. Bio-oil is generally rich in nitrogen, requiring further processing through hydrogenation and catalytic cracking in order to obtain derived bio-oil products (Brennan and Owende, 2013).

1.4 CHALLENGES IN BIODIESEL PRODUCTION

Despite the positive features of 3rd generation biofuels, (see sections 1.2.4 and 1.2.5), several challenges need to be tackled to allow commercial production of algal biodiesel (Scott et al., 2010), competing with the petroleum. Production of microalgal biomass for low value bio-commodity products like energy and fuel remains the most ambitious undertaking of the microalgal industry (Stephens et al., 2013). Consequently, process design and emission inventories will be based on scale-up and assumptions based on knowledge acquired either at lab and pilot-scale or at a microalgae production facility designed for high-value products production (Holma et al., 2013).

As stated in section 1.2.4, some problems affect biodiesel production, making it currently unsustainable. Firstly, algal biodiesel does not compete with petroleum due to its high cost, excluding it from the current liquid fuel market (Hannon et al., 2010). The extraction of the mentioned valuable co-products coupled with biodiesel production could be an interesting challenge decreasing the price gap between algal biodiesel and petroleum (Hannon et al., 2010).

Moreover, a low biomass productivity (low growth rates) and density of algal cells, nutrient and gas utilization, the optimization of lipid content, the complex harvesting and oil extraction procedures are the most important challenges for improving biodiesel production.

Cultivation phase points out different issues to be solved. The first important challenge is the characterization of growth rates and maximum algal cells density in terms of real-world conditions, as explained by

Hannon and coauthors (2010). Laboratory conditions are controlled and they differ to those of a real system. Some parameters such as pH, light supply, temperature and contamination can be checked and monitored while in an open system these conditions can be affected by a large variability i.e. sun light variations. For this reason, the microalgal productivities measured in the laboratory are much greater than those from field results. To date, laboratory production estimates will need to be recalculated for industrially scalable systems (Hannon et al., 2010). After that, these data can be considered meaningful for biodiesel production in both open ponds and PBRs (Hannon et al., 2010).

The second important challenge is improving the use of PBRs and open ponds. As illustrated in section 1.3.1, open ponds and PBR have different characteristics but both of them need to be improved. As explained in section 1.3.1.2, PBRs are more efficient than open ponds for high biomass productivity. Increasing biomass productivity highlights the use of PBR for algal cultivation.

In fact, PBR have the potential to minimize contamination by pathogens and other algal competitor strains but this comes at high capital expense (Hannon et al., 2010) and it will require significant innovations in process optimization (i.e. PBRs design), as illustrated by Stephens and coauthors (2013).

On the other hand, open ponds have lower initial capital costs (Hannon et al., 2010) but algal biomass productivity is low. In fact, another challenge for the industry to increase biomass productivity is the optimization of algal growth in open ponds. Algae growth rates can be limited by light penetration into the ponds from both self-shading and light absorption by the water (Hannon et al., 2010).

Another problem in cultivation phase regards to nutrients supply and their high demand. Nutrients utilization requires high amount of nitrogen and phosphorus. The use of nitrate and phosphate points out some problems, such as high energy requirement for mixing them with water, eutrophication and a large amount of water used for cultivation (Hannon et al., 2010). An interesting and potential solution is the use of wastewater for cultivation. In fact, these nutrients can be supplied by combining nutrient-rich wastewater, streamlining water remediation and optimizing algal growth (Hannon et al., 2010). In these conditions, a few laboratory-based studies suggest that high biomass productivity and lipid accumulation could be reached. For this reason, a real and potential utilization of wastewater for cost-effective biodiesel production (Pittman et al., 2011) could be also considered on commercial scale. Related to this important challenge, the need for efficient and cost-effective algal harvesting must be investigated as well. The small size and a low density of algal cells make the harvesting difficult and costly (Pittman et al., 2011). The lack of efficient algal removal system is the major reason why algal-based wastewater treatment is not used by wastewater industry (Pittman et al., 2011). In fact, both flocculation and centrifugation are energically expensive, even if aluminum flocculation is one of the most used methods for algal harvesting. Harvesting by flocculation is superior to other harvesting technologies because it provides for the treatment of very large quantities of microalgal culture and can be applied to a wide range of species and strains (Uduman et al., 2010). On the other hand, chemical flocculants can affect the performance of anaerobic digestion due to their toxicity (Pedroni et al., 2001). One possible solution could be bio-flocculation influenced by different parameters such as the algal strain, environmental conditions and nutrients stress. The nitrogen stress is favorable to improve bio-flocculation, which requires low energy demand and cost.

In the next years, the improvement of the dual-use microalgae cultivation for wastewater treatment coupled with biodiesel production can reduce nutrients, freshwater, energy cost and GHG emissions (Pittman et al., 2011).

For algae cultivation, the mitigation of CO_2 from flue gas would be ideal in an industrial scenario. To date, a few studies (Douskova et al., 2009 and De Morais et al., 2007) was conducted on laboratory scale and their results point out that the use of waste CO_2 from coal-fired plant can increase algal biomass productivity when CO_2 concentration is lower than 15%.

Given that result, another interesting hurdle is CO_2 capture from industrial emitters (Singh et al., 2010) like chemical plants. Algae can utilize industrial flue gases, removing CO_2, which would otherwise be emitted (Aitken and Antizar-Ladislao, 2012). This solution can reduce high energy costs to produce synthetic CO_2 for algal growth but on a large scale CO_2 distribution could be problematic. In fact, the energy costs of fans used for pump CO_2 into PBR, the capital costs for gas transportation and CO_2 pre-treatment (Scott et al., 2010) could be expensive. As highlighted by Aitken and Antizar-Ladislao (2012), another problem could be a high CO_2

concentration (up to 15%) in the flue gas. This implies an increase of toxicity and other toxins, which can lead to a decrease of algal biomass productivity.

The last important challenge is oil extraction. Both hexane extraction and sCO_2 extraction are expensive, either in terms of equipment or energy required to extract the oil (Hannon et al., 2010). Hexane extraction requires high energy demand for recycling hexane and for drying phase before the oil extraction.

Some studies highlighted that it would be an important advance if methods without drying and solvent extraction could be developed as it would significantly reduce the cost of biomass pre-treatment (Singh et al., 2010). Recent approaches diverge from the conventional approach of dry extraction, and minimize or eliminate these costly processes (Stephens et al., 2013) through different oil extraction methods but there are still challenges in optimizing these processes and issues of capital cost. An investigated method for oil extraction is sCO_2 extraction. Santana and coauthors (2012) stated that sCO_2 extraction has a moderate critical pressure (72.9 bar) allowing for a modest compression cost, while its low critical temperature (31.1 °C) enables successful extraction of thermally sensitive lipid fractions without degradation. sCO_2 extraction facilitates a safe extraction due to its low toxicity, low flammability and lack of reactivity. In addition, drying phase is avoided but it requires high capital costs for the equipment and quite high energy costs, since high pressures must be conducted to bring the solvent into its supercritical state (Kroger and Muller-Langer, 2012). As far as sCO_2 extraction concerns, different improvement has to be reached in order to develop this method on industrial scale for lipid extraction.

All of these problems do not make algal biodiesel production sustainable on commercial scale. Developing pilot scale studies and implementing these technologies in a cost-effective manner could improve algal biodiesel production on industrial scale.

1.5 LIFE CYCLE ASSESSMENT

Life Cycle Assessment (LCA) is a standardized methodology by ISO 14040 and by ISO 14044 (ISO, 2006).

LCA quantifies environmental impacts of a product or a service considering its entire life cycle. This approach is called cradle-to-grave from raw materials extraction to the end of life (Figure 1.11).

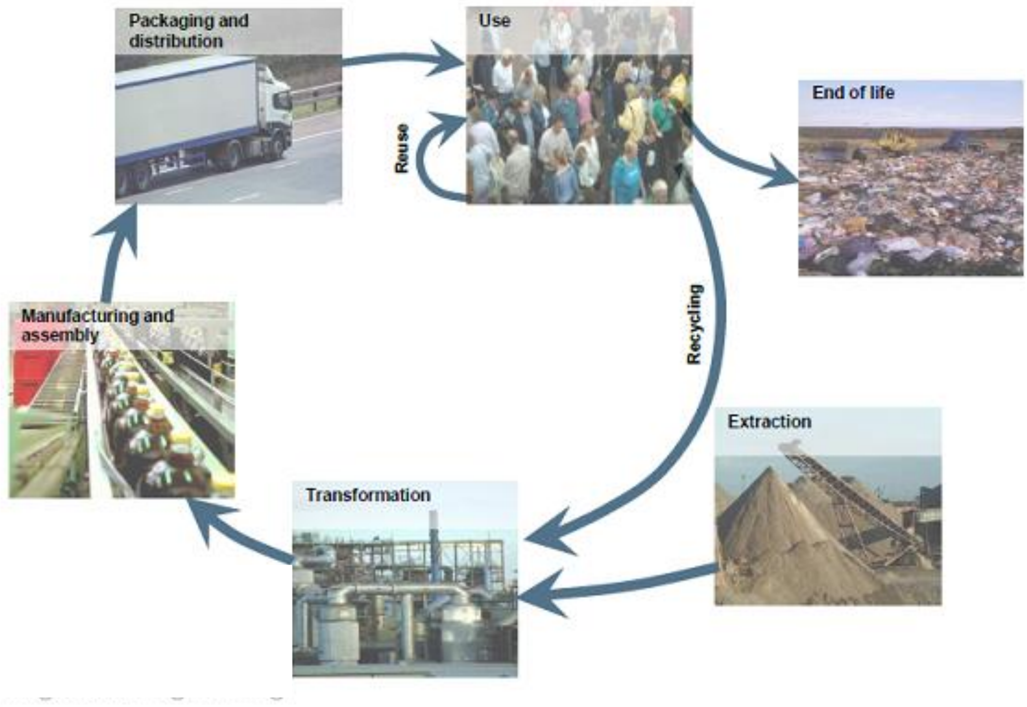


Figure 1.11: Life cycle of a general product

LCA has four phases: goal and scope definition, Life Cycle Inventory (LCI), Life Cycle Impact Assessment (LCIA) and interpretation of results (Figure 1.12).

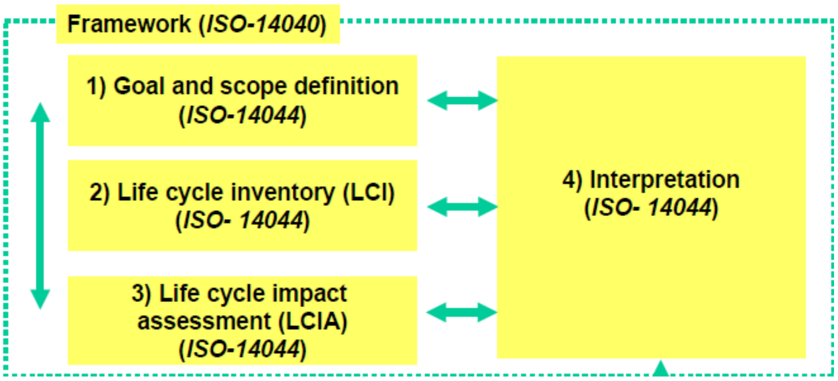


Figure 1.12:LCA phases

1.5.1 Goal and scope definition

ILCD Handbook (EC, 2010) defines the goal definition as the first phase of LCA in order to specify qualitative and quantitative aspects. Among qualitative aspects, decision context and intended applications are defined. The goal definition guides all detailed and quantitative aspects of scope definition. These aspects are functional unit, reference flow, system boundaries, LCI modeling principles (attributional or consequential model), criteria to solve multifunctionality of the system (subdivision, system expansion or substitution or allocation) and cut off criteria.

Functional unit allows the quantification of function of the system. Inputs and outputs are related to functional unit. Reference flow is used to fulfill the function unit.

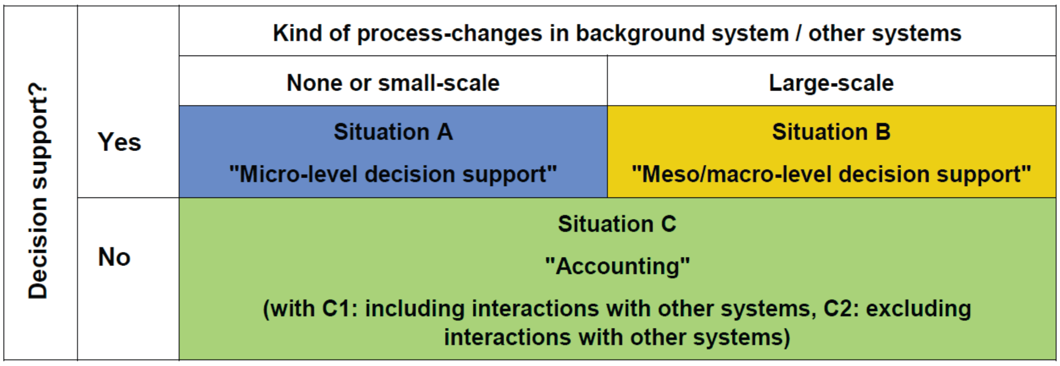
System boundaries are defined as the unit processes considered for the LCA study.

System boundaries can be based on three different approaches: cradle to grave (from raw material extraction to end of life); cradle to gate (from raw material extraction to manufacturing) and gate to gate (considering only production process). Zero burden is related to waste management (only assessing phases after end of life i.e. reuse, recycling, recover). Given that, an explicit goal definition is essential for a correct interpretation of results.

In addition, three decision contexts can be chosen during the goal definition (Figure 1.13).

- situation A ("micro-level decision support"). Decision support is on micro-level, typically for product-related questions. “Micro-level decisions” are assumed to be only limited and no structural consequences outside the decision-context (i.e. do not change available production capacity). The effects are too small to overcome the threshold to be able to cause so called large-scale consequences in the background system or other parts of the technosphere (EC, 2010);
- situation B ("meso/macro-level decision support"). Decision support is in a strategic level (e.g. raw materials strategies, technology scenarios, policy options, etc). “Meso/macro-level decisions” are assumed to have also structural consequences outside the decision-context. The analyzed decision alone results in large-scale consequences in the background system or other parts of the technosphere (EC, 2010);
- situation C ("accounting") is purely descriptive documentation of the system under analysis (e.g. a product, sector or country), without being interested in any potential consequences on other parts of the economy. Situation C has two sub-types: situation C1 that includes existing benefits

outside the analyzed system (e.g. credits existing recycling benefits) and situation C2 that does not do so (EC, 2010).



		Kind of process-changes in background system / other systems	
		None or small-scale	Large-scale
Decision support?	Yes	Situation A "Micro-level decision support"	Situation B "Meso/macro-level decision support"
	No	Situation C "Accounting" (with C1: including interactions with other systems, C2: excluding interactions with other systems)	

Figure 1.13: three different decision context situations (EC, 2010)

In goal definition, data quality and representativeness are also determined to reach a quality control of work. These aspects are assessed in the interpretation of results to achieve conformity with goal definition.

1.5.2 Life Cycle Inventory (LCI)

Referring to ILCD Handbook (EC, 2010), LCI is the second phase to perform a LCA study.

The modeling of the system is done in line with goal and scope definition. Due to the collection of elementary and waste flows, in LCI the highest efforts and resources of LCA are required. After that, inputs are calculated respect to functional unit in order to fulfill a correct and representative system modeling. For each process, data collection must be performed.

Primary data are directly collected from companies. Due to the specificity for some processes, the use of primary data allows more reliability in results.

When it is not possible to obtain primary data from companies, secondary data from literature or from database could be used. In this case, reliability in the results could be affected by less precision and data specificity.

The modeling includes also solving the multifunctionality of processes in the system (EC, 2010).

In LCI, system multifunctionality must be solved in line with the method chosen in goal and scope definition.

As stated in ISO 14044 (ISO, 2006) to carry out the multifunctionality of system, a hierarchy of different methods is followed to avoid allocation:

1. subdivision of system in which the multifunctional process is subdivided into mono-functional sub-processes. For each sub-process, input and output are separately collected.

2. substitution by system expansion of the boundaries for the product system. This means the inclusion of additional functions of co-products.

If allocation cannot be avoided, the inputs and outputs should be partitioned in a way that reflects the physical relationships between them.

3. physical allocation (physical relations are merely the case when a process can be divided according to actions that are only performed due to one of the products).

When a physical relationship cannot be established as the basis for allocation, the inputs and outputs should be partitioned in a way that reflects other types of relationships between them such as mass, economic or energetic value.

1.5.3 Life Cycle Impact Assessment (LCIA)

LCIA is the phase of impact quantification. Inputs and outputs of elementary were collected in the inventory and they are converted into impact indicator results. These results are related to human health, natural environment, and resource depletion.

LCIA identifies the main impact categories and quantifies environmental impact using characterization factor. The LCIA results are calculated by multiplying the individual LCI inventory data with the characterization factor (EC, 2010).

In LCIA, selection, classification and characterization are mandatory steps while normalization and weighting are optional steps in ISO 14044 (ISO, 2006).

First, selection of impact categories and their category indicators must be done. Then, in classification, LCI results are assigned to its impact category. Afterwards, characterization is performed. For each category, characterization factors (CF) are used to calculate the potential environmental impacts. LCI results are multiplied (I) with characterization factors (CF) in order to obtain impact scores (IS).

$$IS=\Sigma_{\mathrm{i}} \left(CF_i \cdot I_i\right)$$

For each substance (i), the indicators are summed to overall category indicators. As LCIA results per impact category have different units, they cannot directly be compared to identify which are the most relevant (EC, 2010).

It is necessary to choose a methodology to express the impact scores. There are different methodologies, based on characterization models. The choice of the characterization model is based on different criteria as environmental relevance, scientific robustness and certainty, documentation, reproducibility, applicability. The choice of LCIA methodology also depends on the goal of the study.

In addition, optional steps can be performed as well.

Normalization aggregates results, dividing each impact score to its normalization factor. Normalization is performed for each impact category.

After normalization, weighting is done using normalized results. As states in ILCD Handbook, weighting involves assigning different quantitative weights to all impact categories in order to express their relative importance (EC, 2010). Weighting factors are used. Weighting results are used for comparison between different impact categories. One problem of weighting is that the weighting factors could be assigned in a subjective way. For this reason, ISO 14044 (ISO, 2006) does not require fulfillment of this step and if weighting was performed, it should be fully transparent.

1.5.4 Interpretation of results

In life cycle interpretation, the LCA results are hence considered collectively. Results are also analyzed to achieve accuracy, completeness and precision of the applied data as well as the check of assumptions. Uncertainty and sensitivity analysis are also performed in this phase. Sensitivity analysis is used to assess which parameters contribute the most to overall impact categories as well as their relevance. On the other hand, uncertainty analysis aims to verify the reliability of the results and conclusions by determining how they are affected by the variations in the hypotheses, methodologies, and data.

1.6 SUSTAINABILITY OF BIODIESEL FROM ALGAE

Sustainable development concept is defined by World Commission on Environment and Development as “the development that meets the needs of the present without compromising the needs of future generations” (UN (United Nations). 1987. Our common future. New York, NY: UN World Commission on Environment and Development). Sustainability considers a balance between economy, environment and society. LCA considers environmental sustainability only.

As stated by Mata and coauthors (2013), different indicators for sustainability of algal biodiesel are identified like energy consumption, net GHG emissions, water and nutrient consumption and land use, etc. For this reason, LCA appears to be a suitable tool to assess the environmental sustainability of algal biodiesel.

In the application of LCA on a biodiesel product system, the functional unit makes it possible to compare the results with the results of reference products. These reference products could be a fossil fuel or biodiesel from 1st and 2nd generation.

LCA also can be used to indicate if the production of biodiesel can lead to negative environmental impacts such as eutrophication, global warming, ozone depletion, human and marine toxicity, land competition and photochemical oxidation.

Additionally, energy balance can be calculated to determine the energy hotspot of all stages within the system boundary of the LCA of biodiesel. For example, it is important to use the energy efficiency ratio (EER), defined as energy output to energy input. If EER has a ratio higher than 1, net positive energy will be reached. This means a positive energy balance and large-scale biodiesel production could be sustainable.

In conclusion, LCA can show which improvements should be done in weak processes of algal biodiesel production in terms of energy and of the environmental impacts.

1.7 STATE OF ART OF LCA FOR BIODIESEL PRODUCTION FROM MICROALGAE

Since the sustainable production of algal biodiesel has been an important research field in recent years, a number of analyses of different technologies and quantification of its environmental impacts have been carried out using LCA.

Depending on goal and scope of the LCA study, many different works have been published giving a general overview about this new research field and about the problems related to it.

One of the main problems about LCA of algae-biodiesel is a lack of experimental data for long term operation of full-scale commercial algae cultivation system (Clarens and Colosi, 2013). Moreover, due to difference in the goal and scope definition, LCA studies of biodiesel production from algae use different functional units, different system boundaries and different modeling assumptions. For this reason, results of algae LCA studies are difficult to compare (Clarens and Colosi, 2013).

Table 1.1 shows 22 works about LCA of biodiesel production from algae.

These 22 works are analyzed in details, as follows.

1. Lardon and coauthors (2009) performed a comparative LCA. An analysis of potential environmental impacts of biodiesel production from

microalgae and an assessment of energy balance were carried out. For energetic balance, the authors used cumulative energy demand (CED). Algal biodiesel was compared to biodiesel from 1st generation and to diesel from fossil fuels. The functional unit was 1 MJ of biodiesel. A cradle to grave approach was used to define system boundaries. System expansion was done for biogas production from anaerobic digestion of algal oil cake. Laboratory scale data were used in order to carry out this study. Four different scenarios were performed: standard and low nitrogen supply coupled with dry and wet extraction, respectively. Among them, the best option had to be compared to other fuels. The authors chose CML 2001 as LCIA methodology considering most of all impact categories. The most impacting processes were the use of fertilizers during the cultivation in open ponds, energy consumption for centrifugation, drying and fuel combustion. The best option was low nitrogen requirement for cultivation and wet oil algal extraction. Eutrophication and land use had lowest impacts and GWP for algal biodiesel was better than soybean, palm oil and diesel, except to rapeseed. Improvement in oil extraction could be sustainable in algal biodiesel production. In fact if wet extraction was carried out, energy requirement for drying would be avoided. Anaerobic digestion of oil cake to produce biogas for electricity and heat could improve environmental performance of biodiesel production.

2. Batan and coauthors (2010) worked on industrial scale performing life cycle energy (net energy ratio). GHG emissions were also assessed. A comparison between diesel, soybean and algal biodiesel was also carried out. The functional unit was 1 MJ of biodiesel; substitution by system expansion was considered and system boundaries were defined with a “well to pump” approach (from cultivation to distribution of fuel). Cultivation in PBR required a large amount of energy for CO_2 pumping as well as solvent extraction with hexane. Allocation of co-product was important in order to have less energy requirements. Concerning to GHG emissions, both algal biodiesel and soybean biodiesel had lower emissions of CO_2 eq. than diesel.

3. Clarens and coauthors (2010) carried out a comparative LCA on a pilot scale. Algal biodiesel was compared to switch grass, corn and canola biodiesel. Cultivation was in open ponds using wastewater. The functional unit was 317 GJ of energy from biomass; a cradle to gate (from cultivation to drying) approach was taken into account. Impact categories studied were energy consumption (MJ), water use (m^3), GHG emissions (kg CO_2-eq.),

eutrophication (kg of PO_4^{3-}-eq.) and land use (ha). Results show that terrestrial crops require less energy and water. For these crops, GHG emissions were better as well. Since the use of wastewater does not require fertilizers, eutrophication was better for algal biodiesel. Moreover, land use was better as well because algae cultivation uses land more efficiently than terrestrial crops.

If CO_2 was recycled (for example from a coal power fired) to be used for cultivation in wastewater, GHG emissions of algal biodiesel would be lower than terrestrial crops.

4. Jorquera and coauthors (2010) compared open ponds, flat and tubular PBR assessing the energy required for both systems. Functional unit was set to 10^5 kg of dry biomass. Net energy ratio (NER) was calculated as a ratio between energy produced and energy requirements. This means that NER lower than one implies that a cultivation system was energy-expensive. NER was lower than one just for tubular PBR. The highest energetic contribution to cultivation was CO_2 pumping. The best option was open ponds although they need high surface to their building. To prevent high energetic costs, the authors stated that coupling open ponds and PBR could have benefits for a positive NER.

5. Sander and Murhty (2010) compared different technologies for harvesting, filter press and centrifugation in terms of energy and GHG emissions. A well to pump approach was considered. Functional unit was 1000 MJ of energy from algal biodiesel. Centrifugation and drying resulted requiring a high amount of energy. If solar light was used for drying, a large amount of energy could be avoided. Due to the bioethanol production as co-product, the system expansion for algal carbohydrate fraction avoided the production of the equivalent amount of corn. Therefore, energy demand was negative (net energy input/energy in functional unit). This suggests that more energy was produced than consumed. Centrifugation had high CO_2 emissions in contrast to filter press. In fact, filter press had negative CO_2 emissions and this implied an avoided impact. Only if co-products were allocated, the achievement of CO_2 emissions saving could be possible. Sensitivity analysis on lipid content was carried out. Finally, the authors found that an increasing of lipid content could decrease energy demand for biodiesel production.

6. Stephenson and coauthors (2010) worked on LCA to assess GWP and fossil fuel requirements (FER). Fossil fuels were also compared to algal

biodiesel. PBR and open ponds were compared as well. This study was carried out on a laboratory scale. A cradle to grave approach was chosen. System expansion was done for algal cake to produce biogas and for glycerol. Economic value for methane was considered while market price has been used for glycerol. 1 ton of biodiesel was chosen as functional unit. LCIA methodology was EDIP 2003. Maintenance was not taken into account in the biodiesel production. Results showed that PBR are worse than open ponds in GWP and in FER. For open ponds, GWP and FER were lower than fossil fuels. Otherwise for PBR, GWP and FER were higher than fossil fuels, due to high contribution of electricity in cultivation. Anaerobic digestion avoided negative impacts for GWP and FER in both systems.

7. Brentner and coauthors (2011) studied different options for biodiesel production. The aim of this study was also to find different design parameter. These parameters were used to assess the most potentially sustainable system for on industrial scale of algal biodiesel production. Functional unit was 10 GJ of biodiesel. A cradle to gate approach was used for determining system boundaries. Transport, capital machinery and combustion of biodiesel were not taken into account. For this study, cumulative energy demand (CED), GHG emissions, water use, eutrophication and direct land use were the main impact categories. Cultivation was done in freshwater. Flat photobioreactors, tubular photobioreactors and open ponds were assessed as technologies for cultivation. Different flocculants and centrifugation were analyzed as well as different extraction methods. Anaerobic digestion and nutrient recycling were taken into account. CED for flat photobioreactors was less than tubular ones and open ponds. Centrifugation was worse than flocculation. High amount of energy for drying was required. Supercritical methanol extraction could save energy requirement but it has not been used for algal oil extraction, yet.

8. Campbell and coauthors (2011) carried out a comparative LCA among algal biodiesel, ultra-low sulfur (ULS) diesel and biodiesel from canola. Algae cultivation was supposed into open ponds. System expansion was done for biogas from anaerobic digestion to produce electricity. The biomass fraction not digested was assumed to be used as fertilizers or animal feed. The approach of system boundaries was cradle to grave excluding facilities and construction. The functional unit was calculated by combustion of fuel in a truck for 1 ton-kilometer (tkm) (0.8 MJ). Sources of

CO_2 for cultivation were different. If CO_2 was a waste flow from company, emissions of GHG would be negative. This result means that algal biodiesel can be better than fossil fuels and canola biodiesel.

9. Collet and coauthors (2011) performed a LCA for biogas production and compared it to fossil fuels, biodiesel and bioethanol. Environmental and energetic impacts were assessed. Functional unit was set to 1 MJ produced by combustion in internal engine combustion. A cradle to grave approach was chosen and substitution by system expansion was done for byproducts. LCIA methodology was CML 2001 considering most of its impact categories. Cultivation in open ponds, centrifugation and recycling of nutrients were evaluated. Combustion was assumed neutral for GWP. A large amount of electricity was resulted for biogas production. For this reason biogas was less competitive than algal biodiesel (abiotic depletion, human toxicity, GWP). In biogas, photochemical oxidant formation was better than algal biodiesel as well as eutrophication and acidification. But algal biodiesel and biogas had less impact than diesel in ozone depletion.

10. Khoo and coauthors (2011) assessed life cycle CO_2 emissions and energy demand for biodiesel production. The base case was managed by ICES (Institute of Chemical and Engineering Sciences) in Singapore on a laboratory scale. Results were also compared to different previous works of Jorquera and coauthors (2010), Stephenson and coauthors (2010), Lardon and coauthors (2009) and Clarens and coauthors (2010). Functional unit was set 1 MJ of biodiesel. System boundaries were defined by cradle to gate approach. Cultivation was performed into PBR coupled with open ponds. Cultivation required high amount of energy as well as hexane extraction. Sensitivity analysis was done in order to obtain an optimistic scenario (lipid 45%). Optimistic and base cases were compared to other works. First, energy demand was analyzed. Biodiesel production in PBR analyzed by Stephenson and coauthors (2010) was better than both cases. On the other hand, biodiesel production in open ponds by Stephenson and coauthors (2010) was worse than both scenarios. Lardon and coauthors (2009) reached a biodiesel production better than ICES base- case but not for the optimistic case. Then CO_2 emissions were assessed. In contrast to optimistic and base scenarios, biodiesel production analyzed by Lardon and coauthors (2009) had a saving of CO_2 emissions.

11. Razon and Tan (2011) worked on a net energy analysis for biodiesel and biogas production. NER was calculated as energy output/energy input.

Two algal strains were compared: *Nannochloropsis sp*. and *Haematococcus pluvialis*. Functional unit was 1 kg of methyl ester from algal oil. System boundaries were defined with a cradle to gate approach and cumulative energy demand was assessed. Filtrate from microfilter displaced freshwater, glycerin replaced glycerin from palm oil and ammonium compounds from biogas displaced ammonium nitrate.

H. pluvialis was cultivated in flat photobioreactor and open raceways. Thickener and micro filter were used for harvesting; bead mill and decanter were used before transesterification. Deficit in energy balance were due to electricity use in bead mill, electricity for photobioreactor and fertilizers for cultivation. NER was 0.4.

Concerning to *Nannochloropsis*, cultivation was performed in open ponds, flocculation was done with aluminum sulphate and drying was carried out. Energy balance was negative and NER was 0.09. This deficit was caused by heat for drying and sewage treatment for biogas effluent. If thickener was totally recycled for cultivation, fertilizers use could be reduced and NER could be 0.12. The authors found that any biodiesel production system assessed was not sustainable.

12. Shirvani and coauthors (2011) performed a life cycle energy and greenhouse gas analysis for biodiesel production. In particular, algal biodiesel was compared to fossil fuels. Due to differences in heat and electricity grid mix, six different countries were assessed. Functional unit was defined as 1 MJ of biodiesel produced by algal oil. The approach used for defining system boundaries was cradle to grave and energetic allocation is carried out for algal oilcake and glycerol. Algal oilcake was used for three different utilizations: combined heat and power unit (CHP), biomass boiler and co-firing coal power plant. Cultivation was done in open ponds using NH_3 and PO_4^{3-} and CO_2 from power plant. Four scenarios were discussed: baseline case (case 1) without use of co-product; in case 2, algal oilcake was used in a CHP plant. In case 3, heat requirements were based on zero carbon energy sources as geothermal energy and in Case 3, a smart utilization of oilcake residues was taken into account. Energetic balance was done considering EBR (energy input/energy output). Baseline case and case 2 had an EBR worse than fossil fuels while EBR for case 3 and Case 3 were better than fossil fuels. Additionally six countries were compared. Brazil and France had an EBR similar to fossil fuel as well as GHG emissions. On the other hand, other countries had EBR and GHG

emissions, which were worse than fossil fuels. These negative results were due to differences in grid mix but also for a different utilization of algal oilcake.

13. Sturm and Lamer (2011) performed an energy evaluation of algal biomass production. This assessment considered cultivation in wastewater, harvesting and drying. Functional unit was 1 ton of biodiesel. Six different scenarios were defined in a way that different technologies for harvesting were coupled with different options for drying. Results highlighted that biofuel production was energetically positive for open ponds with wastewater providing nutrients for algal growth. Centrifugation in harvesting and belt filter press for drying were the worst options for their high energy and heat requirements.

14. Xu and coauthors (2011) carried out an energy balance analysis of algal biofuels. Dry and wet route were assessed. Fossil energy ratio was considered in order to evaluate energy balance. If FER is higher than one, energy output is higher than fossil energy input and a favorable balance is reached. Functional unit was 1 ton of dry biomass (*Chlorella vulgaris*). Energetic allocation for electricity and heat was done. A cradle to grave approach was used for defining system boundaries. Dry route produced biodiesel by transesterification, glycerol and oil cake. Oil cake was used for pyrolysis: bio-char, bio-gas and pyrolysis oil were produced. Otherwise, wet route provided green diesel by hydrotreating. Residues were used for supercritical gasification: CO_2 and aqueous phase were the main products. Aqueous phase contained nutrients that were recycled for cultivation. For both dry and wet route, low nitrogen and standard nitrogen conditions were taken into account as well as conditions with allocation and without allocation. In low nitrogen conditions for wet and dry route, FER was higher than under standard conditions. Due to less energy requirements for lipid extraction, dry route was better than wet route. Best cases for dry and wet route were also carried out achieving a higher FER than basic cases. Biodiesel and green diesel FER were compared to fossil fuels and corn ethanol. Both base cases and best cases had a FER better than fossil fuels and corn ethanol.

15. Yang and coauthors (2011) worked on water footprint and nutrients balance for biodiesel production. The authors determined functional unit as 1 kg of biodiesel. A cradle to grave approach was performed to determine system boundaries. Freshwater, seawater and wastewater with added

nutrients were assessed for cultivation. Water after harvesting could be recycled. This means that water footprint was lower than harvesting without water recycling. Water footprint improved when seawater or wastewater were used for cultivation instead of freshwater. Nutrients use was reduced when water was recycled after harvesting or when wastewater was used for cultivation, due to low requirements of nutrients. In fact, in wastewater nitrogen and phosphorus are already contained without adding nitrate and phosphate. Additionally, algal water footprint was compared to other feedstocks. Except to sugar beet, water footprint for algae was better than other conventional feedstocks.

16. Borkowski and coauthors (2012) performed a comparative LCA among renewable diesel (RD2) by hydrogenation and biodiesel by transesterification. A well-to-pump approach was used for settling on system boundaries. Functional unit was 1 MJ of delivered fuel product. Capital costs for cultivation, extraction and fuel conversion equipment were not taken into account. Urea and single super phosphate were used as nutrients for cultivation. Purified CO_2 and CO_2 as a flue gas were considered. Allocation for purified CO_2 was performed between power plant and fuel generation system (37.8% allocation ratio for RD2 and 40% for biodiesel). Aluminum sulphate was used for flocculation. System expansion for residual biomass was performed as follows. Residual biomass was used in three different ways: for animal feed, for direct combustion to produce electricity and for anaerobic digestion to produce biogas for electricity. Another residual product from anaerobic digestion was digestate which was used for fertilization replacing synthetic fertilizers. Fossil fuel consumptions were high for purification of CO_2, for drying and for nutrients supply. GHG emissions were high for CO_2 supply in cultivation, for harvesting, for drying and for extraction. Hydrotreating required less energy from fossil fuels. For this reason, GHG emissions were low. Due to higher fossil energy requirement and GHG emissions in transesterification, biodiesel production was more than twice as energy intensive as hydrotreating. Considering also anaerobic digestion, primary energy requirements decreased in fact electricity and heat could be used for biodiesel production. This means that electricity and heat from anaerobic digestion avoided external use of electricity and heat. Additionally, FER was used for comparison between different scenarios. CO_2 as flue gas coupled with the use of residual biomass as animal feed showed FER higher

than 1 for RD2 and biodiesel. Instead, GHG emissions were better for CO_2 as flue gas and combustion of residual biomass.

17. Chowdhury and coauthors (2012) assessed environmental performance of algal biodiesel production in an integrating system. GWP, energy demand and water use were the main impact categories evaluated. Functional unit was set to 1 ton of biodiesel. System boundaries were defined with a cradle to gate approach. Construction and maintenance were not included. Substitution by system expansion was done for biogas after oil extraction. Biogas was used for producing heat and digestate was dewatered. Its liquid portion was used to replace nutrients in cultivation. Three scenarios were performed. For each scenario, lipid content varied from 0.4 to 0.7. Base case considered only the production of biodiesel. Scenario without allocation recycled nutrients, water and biogas. Scenario with allocation used biogas from AD for producing electricity and fertilizers for cultivation were replaced by recover of nutrients. Algal growth, nutrients use, harvesting and drying required highest amount of energy. Energy demand decreased when lipid content increased. Considering biogas and nutrients recycled, energy demand and NER were lower than base case. In base case, GWP was higher than in the third scenario. GWP was lower than fossil fuels. Recycling nutrients and using biogas, GWP decreased as well as water demand.

18. Frank and coauthors (2012) evaluated methane and nitrous oxide emissions in biodiesel production. Functional unit was one million BTU (British thermal unit) of biodiesel. A cradle to gate approach was used for determination of system boundaries. Baseline scenario considered anaerobic digestion to recover energy and nutrients. Recovery of nitrogen was 80% in order to displace common fertilizers. Biogas from anaerobic digestion was used in a CHP plant for electricity production. The second scenario was performed using hydrothermal gasification. Cultivation, harvesting and lipid extraction required highest energetic consumptions as well as GHG emissions. Compared to diesel, algal biodiesel showed low fossil energy and a saving of CO_2 emissions. Lipid content and productivity decreased GHG emissions as well as electricity consumption.

19. Sevigne Itoiz and coauthors (2012) worked on a comparative LCA between three different algal species (*A. minutum*, *K. veneficum* and *H. Akashiwo*). For each algal species, outdoor and indoor conditions were evaluated. This study was carried out for a PBR pilot plant. Environmental

impacts and energy balance were calculated in order to highlight weakness and strength of this system. These results could be used for the development of on industrial scale biodiesel facility. Functional unit was 1 kg of dry microalgal biomass. System boundaries took into account algal growth, centrifugation and drying. Oil extraction and transesterification were not taken into account. CML 2001 was used as LCIA methodology as well as CED. Abiotic depletion, acidification, eutrophication, GWP, ozone layer depletion, photochemical oxidation, human toxicity, marine and freshwater ecotoxicity were the main impact categories. A sensitivity analysis was performed increasing lipid content of 10% and decreasing electricity consumption of 50% in each scenario. For each algal strain and for indoor and outdoor conditions, energy balance was negative but indoor was better than outdoor condition. The largest contributions were due to algal growth and construction of PBR. Indoor condition required high inputs of energy to provide light and optimal temperature. For this reason, indoor system required more energy than outdoor condition. Concerning to environmental impacts, outdoor system had lower impacts. Due to highest requirement of energy, algal growth and construction of PBR contributed the most to each impact category. If lipid content was 55% and energy consumption was 88% less than basic scenario, energy balance could be positive. If PBR construction material was replaced with a different type, energy requirement and environmental impact could significantly decrease. An interesting result of this work is the importance of construction material for PBR. In other case studies, the construction of PBR is generally cut off.

20. Soratana and coauthors (2012) worked on a comparative LCA on four conditions in order to assess if algal biodiesel is compliant with Renewable Fuel Standard’s (RFS). This policy implies a reduction of 50% in algal biodiesel’s GHG emissions respect to fossil fuels. This LCA also aimed to identify which processes were the most impacting in biodiesel production. Four scenarios were defined considering two production efficiencies (high and low) and two resource sources (synthetic and waste): HS, LS, HW and LW. High efficiency production had lipid content like 70% instead of 50% for low efficiency production. Functional unit was 1 MJ of bioethanol, a cradle to grave approach was used for system boundaries, co-products and byproducts were not taken into account as well as transportation. TRACI was used as LCIA methodology: GWP, acidification, carcinogens and non-carcinogens, respiratory effects,

eutrophication, OD, ecotoxicity and smog. IMPACT 2002+ was used just for defining non-renewable energy. Basic scenario was LS and the other ones were compared to it. HW showed the lowest impacts while LS contributed the most to all impact categories. LW had higher impacts than HS, except to carcinogens. LS and LW contributed more than HS and HW in GWP. Due to high requirement of energy, harvesting with belt filter contributed the most in different impact categories. High-density polyethylene (HDPE) for PBR contributed the most to carcinogens, non-carcinogens, ecotoxicity and smog formation. Hexane for oil extraction had the highest impacts to OD and carcinogens. The most sensitive parameters were lipid content, energy consumption for harvesting, hexane quantity and system lifetime. These parameters were calculated by sensitivity analysis for 4 impact categories: GWP, eutrophication, OD and ecotoxicity. NER was also investigated. Increasing lipid content, NER was increased as well. NER for algal biodiesel was lower than fossil fuels and 1st generation biofuels. The authors found that GWP for these scenarios were not compliant to RFS's requirements and they were higher than conventional diesel. Improvement of energy requirement for harvesting, higher lipid content and allocation of co-products/ byproducts could improve GWP. This means that RFS's requirement could be achieved only under these conditions.

21. Vasudevan and coauthors (2012) carried out a LCA in order to compare different technologies and which of them affected the most GHG emissions, fossil energy inputs and freshwater consumption. This study also assessed environmental performance of biodiesel production. Freshwater cultivation was in small-scale open ponds system. CO_2 was provided by coal power plant as a waste flow. Oil extraction was done with dry extraction, wet extraction and secretion. Residual biomass was used for anaerobic digestion and biogas was used for producing electricity and heat. Biomass was also used as animal feed. Digest effluent was send as a fertilizer in open ponds. Functional unit was 1 MJ of biodiesel. A "pond to wheel" (until vehicle use) approach was used for the definition of system boundaries. System expansion was done. Energy consumption was higher than energy produced in dry extraction while wet extraction and secretion had positive energy balance as well as GHG emissions. In wet extraction and secretion, drying was avoided. Lipid content was a significative parameter for GHG emissions as well as wastewater instead of freshwater.

GHG emissions were higher for cultivation, harvesting and drying and they were worse than fossil fuels.

22. Weinberg and coauthors (2012) analyzed GHG emissions from algal biodiesel, bioethanol and biogas. A comparative LCA was carried out in order to compare open ponds and flat photobioreactors for these different biofuels. In addition, biodiesel, bioethanol and biogas were compared to diesel, gasoline and natural gas. System boundaries were defined with cradle to grave approach; construction, disposal of infrastructures and transport were cut off. Functional unit was set to 1 MJ of fuel, based on lower heating value. System expansion was done for biogas that was used to supply heat to biofuels system. The remaining part of biogas was allocated for bio-methane upgrading. Cultivation was performed for open ponds and photobioreactors. Main differences were found in electricity requirements to pump CO_2, which was provided by a near power plant. Open ponds required less electricity than PBR. Due to different biofuels production systems, harvesting was carried out in different ways. Energy requirements for bioethanol and biogas were higher than those of biodiesel production. Due to higher electricity requirement to pump CO_2 for PBR, GHG emissions for PBR were worse than those of open ponds. Cultivation in PBR contributed the most to overall GHG emissions while harvesting for open ponds was the highest contribution to overall GHG emissions as well as algal oil extraction.

Bioethanol had higher GHG emissions than biodiesel and biogas because of its lower heating value. In PBR, biodiesel, bioethanol and biogas were worse than diesel, gasoline and natural gas, respectively. On the contrary, open ponds were better than fossil fuels except to bioethanol, which was the worst option among them. This occurred because bioehanol had a lower heating value and for this reasons, less energy was produced by 1 kg of biomass. If biogas was a by-product in biodiesel production, savings of GHG emissions could be shown. Italian electricity mix was based on 80% of fossil fuels energy. Recycling nutrients, higher algae concentration and having a different electricity mix could be significant in order to reduce GHG emissions for biodiesel and biogas from algae.

After this extensive review, some main issues can be discussed.

A) Most of these works evaluated **GHG emissions and energy balance**. Only six studies (Lardon et al. (2009), Clarens et al. (2010), Brentner et al. (2011), Collet et al. (2011), Sevigné Itoiz et al. (2012) and

Soratana et al. (2012)) analyzed environmental impacts like eutrophication, ozone depletion.

B) **Functional units are different** from work to work and they can be divided in 4 groups:

1) energy contained into biofuel (Batan et al. (2010), Clarens et al. (2010), Sander and Murthy (2010), Khoo et al. (2011), Frank et al. (2012), Soratana et al. (2012) and Weinberg et al. (2012));

2) energy from biofuel combustion (Lardon et al. (2009), Brentner et al. (2011), Campbell et al. (2011), Collet et al. (2011), Shirvani et al. (2011), Borkowski et al. (2012) and Vasudevan et al. (2012));

3) amount (kg) of biofuel produced (Stephenson et al. (2010), Razon and Tan (2011), Sturm and Lamer (2011), Yang et al. (2011));

4) amount (kg) of dry algal biomass (Jorquera et al. (2010), Xu et al. (2011), Chowdhury et al. (2012)and Sevigné Itoiz et al. (2012)).

C) **System boundaries are different.** Jorquera and coauthors (2010) considered only cultivation while Sturm and Lamer (2011) and Sevigné Itoiz and coauthors (2012) took into account cultivation, harvesting and drying. Other works examined cradle to gate or cradle to grave approach and in the Table 1.1, system boundaries are shown.

D) **Open ponds and PBR were assessed** separately but in 4 works (Khoo et al. (2011); Sander and Murthy (2010), Batan et al. (2010) and Razon and Tan (2011)), open ponds were coupled with PBR. Jorquera and coauthors (2010), Stephenson and coauthors (2010) and Weinberg and coauthors (2012) compared environmental performance of open ponds and PBR.

E) **All works were developed on laboratory and/or pilot scale**. Only Brentner and coauthors (2011) and Sevigné Itoiz and coauthors (2012) gave some suggestions to achieve biodiesel production on industrial scale.

F) Most of these works assessed **biodiesel production coupled with biogas from residual algal biomass.** System expansion for biogas saves GHG emissions and energy use (Lardon et al. (2009), Batan et al. (2010), Sander and Murthy (2010); Campbell et al. (2011), Khoo et al. (2011), Shirvani et al. (2011), Xu et al. (2011), Borkowski et al. (2012), Chowdhury et al. (2012); Frank et al. (2012), Soratana et al. (2012), Vasudevan et al. (2012) and Weinberg et al. (2012)).

G) The **potential use of wastewater and waste CO_2** from coal-fired plant decreases GHG emissions, eutrophication and energy demand (Lardon et al. (2009), Batan et al. (2010), Clarens et al. (2010), Sander and Murthy (2010), Campbell et al. (2011), Khoo et al. (2011), Sturm and Lamer (2011), Xu et al. (2011), Soratana et al. (2012), Weinberg et al. (2012)).

Abbreviation	Authors	Title	Year	Journal	Functional Unit	System Boundaries	LCA
Lardon et al., 2009	Lardon L., Hélias A., Sialve B., Steyer J., Bernard O.	Life cycle assessment of biodiesel production from microalgae	2009	Environmental Science & Technology.	Combustion of 1 MJ of fuel in a diesel engine	Cradle to grave and cradle to combustion	Comparative LCA
Batan et al., 2010	Batan L., Quinn J., Willson B., Bradley T.	Net energy and greenhouse gas emission evaluation of biodiesel derived from microalgae	2010	Environmental Science & Technology	1 MJ of energy produced	Strain-to-pump (well to pump approach)	Life Cycle Energy and GHG emissions and comparative LCA between diesel and soybean biodiesel
Clarens et al., 2010	Clarens A.F., Resurreccion E.P., White M.A., Colosi L.M.	Environmental life cycle comparison of algae to other bioenergy feedstocks	2010	Environmental Science & Technology	317 GJ of energy based on biomass	Cradle-to-gate	Comparative LCA (switchgrass, corn and canola)
Jorquera et al., 2010	Jorquera O., Kiperstok A., Sales E.A., Embiruçu M., Ghirardi M.L.	Comparative energy life-cycle analyses of microalgal biomass production in open ponds and photobioreactors	2010	Bioresource Technology	100,000 kg of dry algal biomass/year assuming its lipid content as 29.6%	Considering only cultivation	Life cycle energy and comparison between PBR and open ponds
Sander and Murthy, 2010	Sander K., Murthy, G.S.	Life cycle analysis of algae biodiesel	2010	International Journal of Life Cycle Assessment	1000 MJ of biodiesel to pump	Cradle-to-gate considering the transportation	Energy demand and GHG emissions

Stephenson et al., 2010	Stephenson A.L., Kazamia E., Dennis J.S., Howe C.J., Scott S.A., Smith A.G.	Life cycle assessment of potential algal biodiesel production in the United Kingdom: a comparison of raceways and air-lift tubular bioreactors	2010	Energy & Fuels	1 ton of biodiesel	Cradle to grave	GHG emissions, FER and water use and comparative LCA
Brentner et al., 2011	Brentner L.B., Eckelman M.J., Zimmerman J.B.	Combinatorial life cycle assessment to inform process design of industrial production of algal biodiesel	2011	Environmental Science & Technology	10 GJ of biodiesel (supposing the high heat of combustion value is 34 MJ/liter)	Cradle to gate	CED, GHG emissions, eutrophication, water use, land use
Campbell et al., 2011	Campbell P.K., Beer T., Batten D.	Life cycle assessment of biodiesel production from microalgae in ponds	2011	Bioresource Technology	Combustion of enough fuel in an articulated truck diesel engine to transport one ton of freight one kilometre, (tkm) = 0.89 MJ of diesel	Cradle to grave excluding production facilities and its constructions	Comparative LCA for GHG emissions
Collet et al., 2011	Collet P., Hélias A., Lardon L., Ras M., Goy R.A., Steyer J.	Life-cycle assessment of microalgae culture coupled to biogas production	2011	Bioresource Technology	1 MJ produced by combustion in an internal combustion engine.	Cradle to grave (combustion of methane)	Comparative LCA for GHG emissions
Khoo et al., 2011	Khoo H.H., Sharratt P.N., Das P., Balasubramanian R.K., Naraharisetti P.K., Shaik S.	Life cycle energy and CO_2 analysis of microalgae-to-biodiesel: Preliminary results and comparisons	2011	Bioresource Technology	1 MJ of biodiesel high heating value	Cradle to gate	Comparative LCA for GHG emissions and energy demand

Razon and Tan, 2011	Razon L.F., Tan R.R.	Net energy analysis of the production of biodiesel and biogas from the microalgae: *Haematococcus pluvialis* and *Nannochloropsis*	2011	Applied Energy	1 kg of algal methyl ester	Cradle to gate	Net energy analysis
Shirvani et al., 2011	Shirvani T., Yan X., Inderwildi O.R., Edwards P.P., King D.A.	Life cycle energy and greenhouse gas analysis for algae-derived biodiesel	2011	Energy and Environmental Science	1 MJ of biodiesel produced from algae-oil	Cradle to grave	Comparative LCA for GHG emissions and energy demand
Sturm and Lamer, 2011	Sturm B.S.M. and Lamer S.L.	An energy evaluation of coupling nutrient removal from wastewater with algal biomass production	2011	Applied Energy	1 ton of biodiesel	Cultivation, harvesting, drying	Energy evaluation of algal biomass production
Xu et al., 2011	Xu L., Brilman D.W.F., Withag J.A.M, Brem G., Kersten S.	Assessment of a dry and a wet route for the production of biofuels from microalgae: energy balance analysis	2011	Bioresource Technology	1 ton of dry algal biomass of Chlorella Vulgaris	Cradle to gate	Comparative Life cycle energy
Yang et al., 2011	Yang J., Xu M., Zhang X., Hu Q., Sommerfeld M., Chen Y.	Life-cycle analysis on biodiesel production from microalgae: water footprint	2011	Bioresource Technology	1 kg di microalgae to produce biodiesel	Cradle to gate	Water footprint
Borkowski et al., 2012	Borkowski M.G., Zaimes G.G., Khanna V.	Integrating LCA and thermodynamic analysis for sustainability assessment ofalgal biodiesel.	2012	Sustainable Systems and Technology (ISSST),	1 MJ of delivered fuel product	Well-to-pump	Comparative LCA for GHG emissions and energy demand

Chowdhury et al., 2012	Chowdhury R., Viamajala S., Gerlach R.	Reduction of environmental and energy footprint of microalgal biodiesel production through material and energy integration	2012	Bioresource Technology	1 ton of algal biomass	Cradle to gate	Comparative LCA for GHG emissions, energy demand and water use considering differen technologies
Frank et al., 2012	Frank E.D., Han J., Palou-Riviera I., Elgowainy A.	Methane and nitrous oxide emissions affect the life cycle analysis of algal biofuels	2012	Environmental Research Letters	1 milion BTU of diesel	Cradle to grave	Comparative LCA
Sevigné Itoiz et al., 2012	Sevigné Itoiz E., Fuentes-Grunewald C., Gasol C.M., Garces E., Alacid E., Rossi S., Rieradevall J.	Energy balance and environmental impact analysis of marine microalgal biomass production for biodiesel generation in a photobioreactor pilot plant	2012	Biomass and Bioenergy	1 kg of dry microalgal biomass from each species studied	Cultivation, harvesting, drying	Energy balance
Soratana et al., 2012	Soratana K., Harper Jr. W. F., Landis A. E.	Microalgal biodiesel and the renewable fuel standard's greenhouse gas requirement	2012	Energy Policy	1 MJ of bioethanol	Cradle to grave	Comparative LCA
Vasudevan et al., 2012	Vasudevan V., Stratton R., W., Pearlson M.N., Jersey G.R., Beyene A.G., Weissman J.C., Rubino M., Hileman J.I.	Environmental performance of algal biofuel technology options	2012	Environmental Science & Technology	1 MJ of biodiesel	Pond to wheel	NER and GHG emissions. Comparative LCA for between wet, dry and secretion
Weinberg et al., 2012	Weingberg J., Kaltschmitt M., Wilhelm C.	Analysis of greenhouse gas emissions from microalgae-based biofuels	2012	Biomass Conversion and Biorefinery	Amount of fuel which corresponds to the energy content based on the lower heating value in MJ	Cradle to grave excluding transportation	Comparative LCA

Table 1.1: state of art on LCA for biodiesel production from microalgae. Abbreviation found in this work, authors, title of literature reference, journal, functional unit, system boundaries and LCA type are described in this table

2 THE CASE STUDY: PRODUCTION SYSTEM OF BIODIESEL FROM MICROALGAE

The aim of this work is to provide a hypothetic model about biodiesel production on industrial scale, using the available technologies and focusing on which parts must be improved.

In the previous section 1.3, a general overview highlighted the main processes of biodiesel production. Technologies adopted and main phases considered can be described as follows.

In this case study, cultivation, harvesting, drying, oil extraction and transesterification are assessed as well as anaerobic digestion for residual biomass and glycerol used for glycol propylene.

Flat panel photobioreactors (PBR) are assumed to be used for microalgae cultivation. Each PBR is 2.5 m long, 1.5 m high and 0.070 m thick. Each PBR volume is 263 m^3. The number of FPBR per hectare is 2666.67 (Brentner et al., 2011). For FPBR, construction materials are low density polyethylene (LDPE) sheets (20266.667 kg/ha) and steel (1600 kg/ha) (Figure 2.1). Their construction phase has a lower impact than their operating phase (see section 5.2.1)

Figure 2.1: Flat Panel Photobioreactor

In their work, Jorquera and coauthors (2010) estimated that *Nannochloropsis* has a productivity of 0.27 kg/m^3/day, a lipid content of 29% (Rodolfi et al., 2009) and a biomass productivity of 37.8 t/ha/y. The cultivation takes place in Denmark and 200 productivity days are considered. Geographical location and solar irradiation must be considered because they affect the amount of productivity. In this case, solar irradiation is 3,730,000,000 J/m^2/y for Denmark (Danish Meteorological Institute, 2012).

In addition, CO_2, nutrients like ammonium nitrate and mono calcium phosphate are added to water.

The second phase is flocculation. For algal harvesting, this process can be achieved with aluminum sulphate, lime (

Figure 2.2) or centrifugation. The description of technologies was performed in chapter 1.3.2.

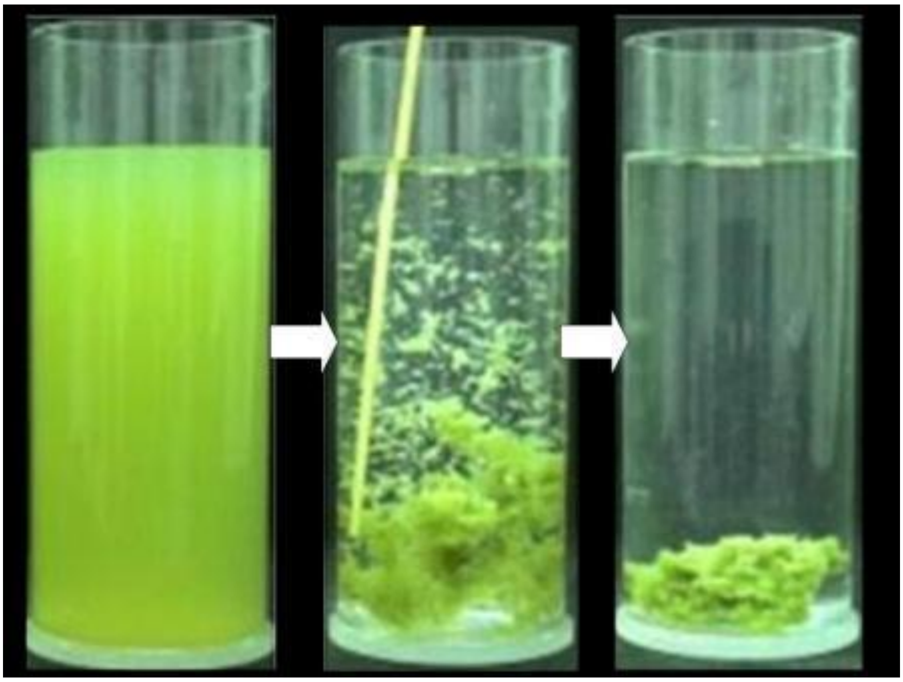


Figure 2.2: an example of flocculation. Algal biomass is suspended in the water. After flocculation, algal biomass can be dried

In oil extraction, two different methods are alternatively investigated:

1) press, oil extraction and transesterification. This process starts with the drying of algal biomass to obtain dry biomass. Algal oil is extracted by dry biomass with the use of hexane. Finally, transesterification with methanol is used for the production of biodiesel.

2) supercritical CO_2 extraction and transesterification. In this method, drying step is avoided. Wet algal biomass is directly used for algal oil extraction. Supercritical CO_2 extraction is performed. This is a selective process for lipid extraction. For algal oil extraction, optimal conditions are 300 MPa and 100°C (Brentner et al., 2011). After that, biodiesel is produced by transesterification.

In addition, residual biomass from oil extraction is used to produce biogas with anaerobic digestion. This biogas is used to produce electricity.

In the transesterification, glycerol is a by-product and it is mainly used for the production of glycol propylene.

3 LCA MODELLING ON PRODUCTION OF BIODIESEL FROM ALGAE

The goal of these sections is implementing an LCA model of biodiesel production from algae. Secondary data from literature are used. Results are used to assess the environmental sustainability of biodiesel production on industrial scale.

3.1 Goal definition

The goal definition is the first phase of any life cycle assessment, independently if the LCI/LCA study is limited to the development of a single unit process data set or it is a complete LCA study of a comparative assertion to be published (EC, 2010).

In order to define the goal of this LCA, five points must be followed. The goal is to assess the production system of algal biodiesel. This means an analysis about its environmental performance, its hot spot and the technologies used in the system.

3.1.1 Intended applications

Firstly, the goal definition shall **state the intended applications of the LCA results** in a precise and unambiguous way (EC, 2010).

The intended application of this study is an analysis of weak points for biodiesel production. This analysis aims to emphasize environmental and energetic impacts as well as a comparison among different technologies for each phase. In addition, the relationship between environmental impacts and technologies is investigated.

3.1.2 Method, assumption and impact limitations

As stated by Clarens and Colosi (2013), one of the main limitations of LCA study about algal biodiesel is a experimental data lack for each process. As a result of this issue, none of the studies analyzed in the section 1.7 was carried out on industrial scale. Brentner and coauthors (2011) and Sevigné Itoiz and coauthors (2012) worked to develop biodiesel production on industrial scale. Consequently, in this study, a lack of empirical data on industrial scale is a relevant limitation for algae cultivation systems. For this reason, many assumptions are made in the model.

3.1.3 Reasons to carrying out the study

In this case study, the reasons to perform an LCA are:

1. environmental assessment of algal biodiesel production;

2. improving biodiesel production on industrial scale identifying the most sustainable technologies for this system.

3.1.4 Decision context

As described in section 1.5.1, a decision context must be chosen. In relation with intended applications, the decision context is situation A (EC, 2010). The situation A is for a decision support. This situation assumes that decision will only cause changes that are too small to affect background and market mechanisms. The consequences are on a small and in the foreground system.

Situation A also covers the development of LCIA data that are used in LCA-based decision support (EC, 2010). This study will not point out in a long term period but only on a small scale and for a micro-level support decision.

3.1.5 Target audience and commissioner of the study

The results are intended to be communicated but not be disclosed to public. Target audience could be industrial company, university researchers or industrial laboratories. For this reasons, the type of target audience could be external and technical.

3.2 Scope definition

In the scope definition, the object of LCI/LCA study is identified and defined in detail. This shall be done in line with the goal definition (EC, 2010).

3.2.1 Function, Functional Unit, Reference Flow

The system function is related to the product and its use. In this case, the primary function of the system is the biodiesel production. The primary function of biodiesel is being a fuel used for combustion in a diesel engine. Therefore, this function presents some qualitative aspects. They are obligatory and positioning properties. Obligatory and positioning properties are used for quantification of the function.

The obligatory properties of biodiesel identify some biodiesel features. For example: good cold properties, good ignition point, low viscosity, medium density, no sulfur content, low degree of unsaturation and a low presence of free fatty acids. On the other hand, the positioning property is to be rich in additive chemicals. This prevents some problems as crystallization or wax formation.

Biodiesel function must be quantified with the functional unit. Supposing a high heating value (HHV) as 39.35 MJ/kg (Brentner et al., 2011), the functional unit of this study is 1 MJ of biodiesel.

It is recommended to use the energy produced by biodiesel rather than the quantity of biodiesel produced. This functional unit allows comparison with other kind of fuels or biofuels.

The reference flow is a quantitative expression of the amount of product, which must be provided by the product system. All input and output of the system are quantitatively related to this flow. In this case, the reference flow is 1 MJ of biodiesel.

3.2.2 LCI modeling framework and LCI method approaches to solve multifunctional process

There are two different situations to model a system: attributional and consequential.

The attributional life cycle models represents its actual or forecasted specific or average supply-chain, its use and end-of-life value chain. The existing or forecasted system is embedded into a static technosphere (EC, 2010).

In the case study, the attributional model is chosen due to the consideration of potential impacts. Those impacts can be attributed to biodiesel production system, using specific data for main processes and average data for other ones.

In contrast, consequences of environmental impacts on the market are not assessed (consequential LCA).

Biodiesel production is a multifunctional process: the main product is biodiesel. Anaerobic digestion of residual biomass produces a main co-product: biogas. Biogas can be used to provide electricity from a cogeneration plant. This electricity avoids the use of the same quantity of electricity from Danish production mix.

Additionally, the main byproduct from transesterification of algal oil is glycerol. Algal glycerol is mainly used to produce propylene glycol which is the most economically attractive for chemical industry (Jorgensen et al., 2012). Hence, the use of propylene oxide is avoided and replaced by glycerol.

In line with ILCD Handbook, allocation is the last option used to solve multifunctionality. Both biogas and glycerol are used in other systems. According to ILCD Handbook, if the secondary function (biogas and glycerol) acts within another system, substitution by system expansion is performed. Substitution by system expansion is applicable for attributional model including existing interactions with other systems to avoid primary production. Substitution means the subtraction of the inventory of another system from the analyzed system. In some cases, this leads to negative inventory flows and it can even result in negative overall environmental impacts for analyzed system (EC, 2010).

In this case, Danish production mix for electricity and propylene oxide are avoided and replaced by the use of biogas and glycerol.

3.2.3 System boundaries

System boundary defines which processes or activities belong to the product system, i.e. they are required for providing the function as defined in the functional unit. The system boundaries should be represented in a semi-schematic flow-chart type diagram. This diagram shows which parts of the product system are included and which are excluded.

In this case study, the approach used to define system boundaries is “cradle to gate”. This approach considers each phase of the process starting from cultivation to transesterification. Transportation and biodiesel use are not taken into account. Biodiesel production system is shown in Figure 3.1

In system boundaries, the definition of foreground and background processes is also important. The foreground system considers those processes of the system which are specific to it. On the other hand, the background processes are typically represented by average data which represent the relevant mix of technologies (e.g. for materials or electricity) (EC, 2010). In the system analyzed, foreground processes are:

- cultivation;
- harvesting;
- drying;
- oil extraction;
- transesterification (biodiesel production);

The background processes are the electricity and heat use.

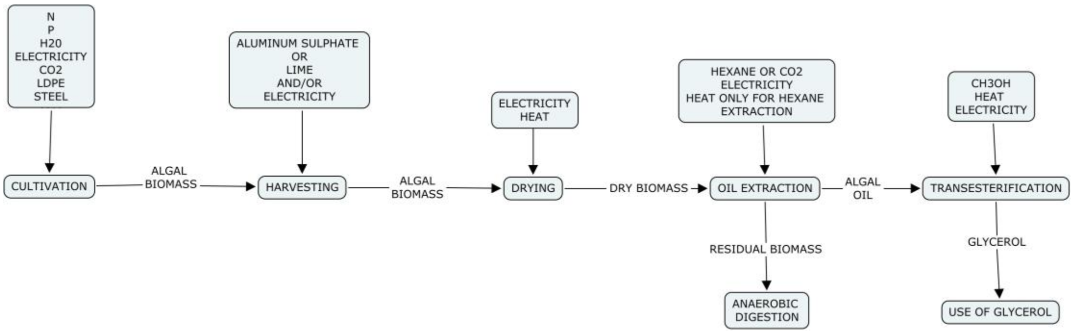


Figure 3.1: System boundaries of biodiesel production

3.2.4 Cut-off criteria

In general, all processes and flows that are attributable to the analyzed system must be included in the system boundaries. Cut-off refers to the omission of not relevant life cycle stages, activity types, specific processes, products and elementary flows from the system model. At the beginning, the choice of cut-off is arbitrary and then it can be improved after different iterations. It is the minimum percentage by mass, which is used to exclude the flows that have significance less than the cut off. Below this percentage, environmental impacts of these flows are not considered. A stream with small contribution and high environmental impact should be taken into account. In fact its exclusion could be a problem in the LCIA.

Manufacturing and use of infrastructures are not included in the LCIA because they could not be relevant for biodiesel production. Maintenance of facilities is not included as well.

3.2.5 Scenario

The goal of this section is a combination of different technologies in order to work with different scenarios. Since biodiesel has not been produced on commercial scale yet, the performed scenarios are hypothetic for implementing a system as real as possible.

In this work, six scenarios are analyzed. Their main differences are in the use of different technologies for harvesting and for the oil extraction. Each scenario presents pros and cons. Scenarios and their limitations are described as follows:

1. scenario “aluminum and hexane” (scenario 1: al., hex.). In this scenario, aluminum sulphate is used as flocculant and the extraction method is based on solvent extraction, in which hexane is used. One of the main limitations is aluminum sulphate toxicity which can affect some impact categories.

2. scenario “lime and hexane” (scenario 2: li., hex.). In this scenario, lime is used as flocculant and the extraction method is based on solvent

extraction, in which hexane is used. The most important limit of this scenario is lime flocculation that has not been developed enough to algal biomass harvest. However, this flocculation method could be developed because lime is less toxic than aluminum sulphate.

3. scenario “centrifugation and hexane” (scenario 3: centr., hex.). In this scenario, centrifugation is used as harvesting technology and the extraction method is based on solvent extraction, in which hexane is used. The main bottleneck is a large energy demand for centrifugation.

In the subsequent scenarios, limitations of harvesting are the same as those of the scenarios described above. In the new scenarios, supercritical CO_2 extraction could be an interesting technology, even if it requires high capital costs. It is an innovative oil extraction method for algal biomass. In this work, it is performed and compared to hexane extraction in order to point out its strengths and weakness.

4. scenario “aluminum and supercritical CO_2 extraction (sCO_2)” (scenario 4: al., CO_2). In this scenario, aluminum sulphate is used as flocculant and the extraction method is based on the use of CO_2 in its supercritical conditions (27.5 MPa and 47.5°C, Mendes and coauthors (1995)) without considering drying before.

5. scenario “lime and supercritical CO_2 extraction” (scenario 5: li., CO_2). In this scenario, lime is used as flocculant and the extraction method is based on the use of CO_2 in its supercritical conditions (27.5 MPa and 47.5°C, Mendes and coauthors (1995)) without considering drying before.

6. scenario “centrifugation and supercritical CO_2 extraction” (scenario 6: centr., CO_2). In this scenario, centrifugation (without flocculant) is used as harvesting technology and the extraction method is based on the use of CO_2 in its supercritical conditions (27.5 MPa and 47.5°C, Mendes and coauthors (1995)) without considering drying before.

For each scenario, both freshwater (fresh) and a hypothetic use of wastewater (waste) are considered as well as synthetic CO_2 (a flue gas produced only for algae cultivation) and waste flow CO_2 coming from a Danish cement industry. For this reason, 24 hypothetical scenarios are analyzed. “Freshwater scenarios” assume the use of tap water in which nutrients like ammonium nitrate and phosphate are added for algal growth. “Wastewater scenarios” assume the use of wastewater for algal growth. the use of wastewater avoids the addition of nutrients due to its enrichment in nitrogen and phosphorus.

The use of waste CO_2 is implemented on laboratory scale but it could be developed on industrial scale for CO_2 mitigation (see section 1.4).

As explained in section 1.7, biodiesel is coupled with biogas from a residual biomass fraction. Given that consideration from literature review, all scenarios take into account biogas production from anaerobic digestion.

The literature analysis points out that aluminum flocculation and hexane extraction with drying phase are the most used technologies. For this reason in the section 5.2, results will be analyzed for scenario “aluminum and hexane” because it is considered a basic scenario.

In this analysis, “freshwater and wastewater scenarios” with both synthetic CO_2 and waste CO_2 will be investigated. In addition, scenario “aluminum and sCO_2” will be analyzed as well in order to highlight the main differences respect to scenario 1 in terms of environmental performance.

Additionally, in appendix 9.1 all results will be summarized.

3.2.6 Preparing the basis for the impact assessment

In LCIA, a selection of impact categories must be comprehensive and cover all relevant environmental issues related to the analyzed system. LCIA methods exist for midpoint and for endpoint level, and for both in integrated LCIA methodologies. In general, on midpoint level, a higher number of impact categories is chosen. For this reason, results are more accurate and precise.

Main impact categories at midpoint and at endpoint are shown in Figure 3.2.

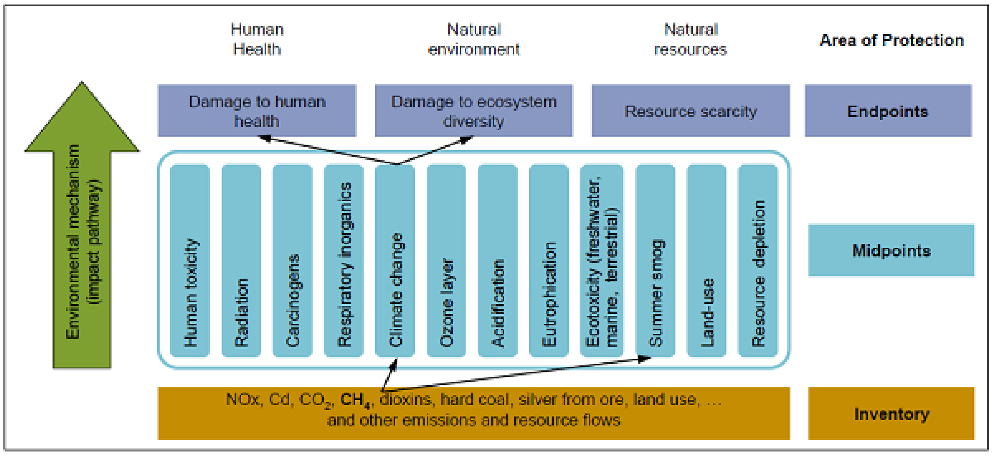


Figure 3.2: midpoint and endpoint level of impact categories (EC, 2010)

In biodiesel production, the overall impact categories are taken into account. This process has several impacts which can affect different environmental media.

An assessment of the energy use is also performed. For this reason, non renewable energy consumption is evaluated.

LCIA method chosen is IMPACT 2002+ and nine impact categories are considered. These categories are:

- aquatic eutrophication (EP);
- aquatic acidification (AP);
- carcinogens;
- global warming potential (GWP) (horizon time is 500 years);
- non carcinogens;
- non renewable energy consumption;
- photochemical oxidation (POCP);
- ozone depletion (ODP);
- terrestrial acidification (AP).

3.2.6.1 Aquatic eutrophication

The enrichment of waters by nutrients (nitrogen and phosphorus) increases the growth of algae and plants in the water to the extent that they smother and suffocate wildlife. Due to the use of fertilizers, agriculture is a relevant source of nitrogen and phosphorus but also wastewater treatment plants for households and industry play an important role. Characterization factors are calculated with an assessment of the number of moles of nitrogen or phosphorus which can be released into the environment by one mole of the substance emitted. EP is expressed by kg of PO_4^{3-} eq.

3.2.6.2 Aquatic and terrestrial acidification

H^+ release into water and soil cause a decreasing of pH. This leads to acidification for water and for soils. The main responsible compounds of acidification are SO_X and NO_X from fossil fuels combustion.

Characterization factors are calculated as follows:

$AP_i=\eta_i /\eta_{SO2}$

where η_i (mol/kg) is the number of H^+ moles that can potentially be produced per 1 kg of substance $_{i;}$ η_{SO2} (mol/kg) is the number of H^+ moles that can potentially be produced per 1 kg of SO_2. For this reason, aquatic and terrestrial AP is expressed in kg of SO_2-eq.

3.2.6.3 Human toxicity: carcinogens and non carcinogens

These impact categories are related to human toxicity (HT). Characterization factors for toxicity are different for each methodology. The model for toxicity characterization factors is

CF_i =FF x XF x EF

where FF is fate factor, XF is exposure factor and EF is effect factor.

In IMPACT 2002+, carcinogens and non carcinogens are expressed in kg of C_2H_3Cl eq.

3.2.6.4 GWP

Due to increasing of human activities and use of fossil fuels, greenhouse gases (GHG) increases in atmosphere. This causes global warming. Earth surface’s temperatures increase every year. The Intergovernmental Panel on Climate Change (IPCC) defines climate change as “a change in the state of the climate that can be identified by changes in the mean and/or the variability of its properties, and that persists for an extended period, typically decades or longer”. Climate change is measured with its indicator of category, the radiating force. The radiative force values are for changes relative to preindustrial conditions defined at 1750 and are expressed in watts per square meter (W/m^2) (IPCC, 2007). GWP is used to calculate the characterization and period of the radiating force generated by one kg of the gas immediately injected into the atmosphere. Characterization factor of GWP is GWP. It is expressed by kg of CO_2 eq. and it is calculated as follows.

$$GWP_i = \frac{\int_0^T a_i . C_i(t).dt}{\int_0^T a_{CO_2} . C_{CO_2}(t).dt}$$

where:

- a_i: radiating force following an increase of one unit in the concentration of gas_i;
- aCO_2: radiating force referred to CO_2, expressed by W/m^2
- $C_i(t)$: concentration of gas_i remaining at time t after emission;
- C_{CO2}: CO_2 concentration of CO_2 remaining at time t after emission;
- T: number of years for which the integration was carried out. In IMPACT 2002+, horizon time corresponds to 500 years.

3.2.6.5 Non renewable energy consumption

Characterization factors for non renewable energy consumption are based on upper heating value. Non renewable energy consumption is expressed by the total primary extracted. For this reason, non renewable consumption is assessed in MJ.

3.2.6.6 Photochemical oxidation

In photochemical oxidation, volatile organic compounds react with ozone, forming tropospheric ozone and other photo-oxidant compounds. This reaction is catalyzed by solar irradiation. This is due to the combustion of fossil fuels that produces nitrogen oxides (NO_X). POCP is expressed by kg of C_2H_4 eq.

3.2.6.7 Ozone depletion

Due to human activities, ozone depletion is caused by an increasing of CFC and HCFC emissions in the atmosphere. This implies an increasing exposure of UV radiation for Earth’s surface.

Characterization factors (ODP) are calculated by World Meteorological Organization (WMO), as follows:

$ODP_i = \delta[O_3]_i/\delta[O_3]_{CFC\text{-}11}$

where CFC-11 is considered a reference substance. Ozone depletion is expressed by kg of CFC-11 eq.

3.2.7 Representativeness and appropriateness of LCI data

In an attributional model, the use of real data represents the supply chain. The technical representativeness regards specific data for supply chain of biodiesel production. The choice of each process is based on the best available technology in biodiesel production. In order to compare new technologies and choose the best option among those explained in section 3.2.5, different scenarios are performed.

Concerning to geographical representativeness, it is necessary identifying how well the inventory data represent their location. For every step, assumptions are made about the processes and where they take place. It is assumed that the overall biodiesel production takes place in Denmark.

In cultivation and harvesting, the geographical representativeness is relevant because each country has different weather conditions. These conditions can influence algal growth and their harvesting. Drying, oil extraction and transesterification take place in Denmark as well.

Energy consumption is based on Danish electricity production mix (Ecoinvent 2.0). Heat consumption is based on heating mix, using in European country. Due to lack of specific flows for Denmark, other flows (water, carbon dioxide, nutrients, hexane and methanol) are related to Europe. Using European average data, average features are shown avoiding the choice of specific and regional processes that could have replaced Denmark.

Biodiesel production is still developing and therefore the data should be recent. In Ecoinvent, flows refer to 10 years ago at least. This means a quite low temporal representativeness. In contrast, data used for inventory are recent referred to the last 4 years (from 2009).

Every process data set represents the true process of the system. The use of some assumptions can improve appropriateness of the data set. In this way, consistency of data used is developed and improved as the best available for biodiesel system production.

3.2.8 Types, quality and sources of required data and information

As far as possible it should be aimed to obtain specific data of production system directly from companies or producer (Olsen et al., 2012).

Initially, a research of main Danish and European companies producing algal biodiesel was done. This is for a collection of primary data on industrial scale.

Due to the current development and improvements of biodiesel from microalgae, data provided by companies or direct producers of algal biodiesel are not available. This means that collecting data of the overall process is not simple. For this reason, only secondary data are used to model the production system. Data are collected using as main reference the article “Combinatorial Life Cycle Assessment to inform process design of industrial production of algal biodiesel” written by Brentner and coauthors (2011). The object of this article is the identification of design parameters that collectively indicate the most potentially sustainable system for industrial-scale production of algal biodiesel (Brentner et al., 2011). Some data, for example waste flow of CO_2 from Danish cement industry, solar irradiation, and Danish electricity mix, are calculated using Danish parameter. Then, these data are adapted to data from Brentner and coauthors (2011). On the other hand, other data are directly calculated by this literature reference. This is due to the lack of data in this research matter.

A few literature articles are used to develop the data set used in this case study:

- “Life Cycle energy and CO_2 analysis of microalgae-to-biodiesel: preliminary results and comparisons” by Khoo and coauthors (2010);
- “Life Cycle Assessment of biodiesel production from microalgae” by Lardon and coauthors (2009);

- “supercritical CO_2 extraction of carotenoids and other lipid from *Chlorella vulgaris*” by Mendes and coauthors (1995).

Afterwards, a further lack of data which cannot be solved by literature references and a less complex modeling of some aspects of biodiesel production are settled through the use of several assumptions, consistent with the overall system analyzed.

4 LCI INVENTORY ANALYSIS

During the life cycle inventory phase, data collection and modeling of the production system must be done. This is to be performed in line with the goal and scope definition. The LCI results are the input to the subsequent LCIA phase (EC, 2010).

4.1 Collecting data

LCI data are collected and splitted in each phase for biodiesel production (cultivation, harvesting, drying, algal oil extraction, anaerobic digestion and transesterification). Every phase in biodiesel production consists in a process unit. They are linked each other by intermediate flows and linked to environment by elementary flows. For each process unit, different energy and mass flows are collected. Their outputs are elementary flows as well and they are considered as emissions to the environment.

Data used for inventory are described in the following tables (Table 4.1, Table 4.2, Table 4.3, Table 4.4, Table 4.5, Table 4.6 and Table 4.7). Each of these tables represent a process in the biodiesel production chain.

Amount of flows and their related process in Gabi are shown as well as which database is used and the source of literature references. Comments about process or assumptions are made as well.

4.1.1 Data for cultivation

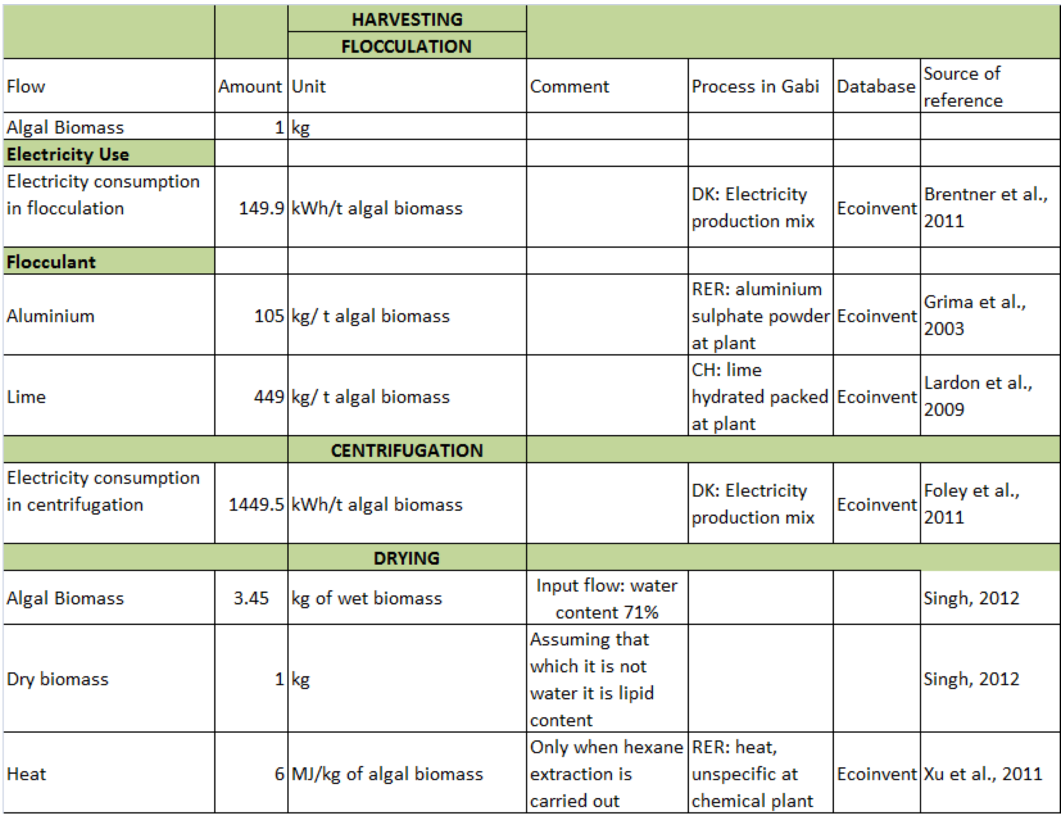
Input for freshwater and wastewater cultivation are illustrated in the Table 4.1. In freshwater, nitrate and phosphate are synthetic nutrients. For this reason, these nutrients are added in tap water while wastewater is already rich in nitrogen and phosphorus. This means that synthetic nutrients for algal growth are not added in wastewater. Wastewater is used after its sewage treatment while freshwater is properly produced and used for algal cultivation. Other input amounts are the same for both options.

	FLOWS USED FOR BIODIESEL PRODUCTION					
	FRESHWATER CULTIVATION					
Flow	Amount	Unit	Comment	Process in Gabi	Database	Source of reference
Algal Biomass	1	kg				
Carbon dioxide (CO_2)	1790	kg/t algal biomass	emission from cement industry	RER: carbon dioxide liquid at plant	Ecoinvent	Sturm and Lamer, 2011 and Wijffels and Barbosa, 2010
Water	1424.5	m^3/t algal biomass		RER: tap water at user	Ecoinvent	Brentner et al., 2011
Electricity Use						
Aeration CO_2	1963.0	kWh/t algal biomass				
Outflow pumping H_2O	325.9	kWh/t algal biomass				
Total electricity consumption in cultivation	2288.9	kWh/t algal biomass		DK: electricity production mix	Ecoinvent	Brentner et al., 2011
Material used for FPBR						
LDPE sheet	10.7	kg/t algal biomass		RER: polyethylene LDPE, granulate at plant	Ecoinvent	Brentner et al., 2011
Steel	0.8	kg/t algal biomass		RER: reinforcing steel at plant	Ecoinvent	Brentner et al., 2011
Nutrient use						
Ammonium nitrate	234.3	kg/t algal biomass	35% of nitrogen in freshwater cultivation	RER: ammonium nitrate, as N, at regional storehouse	Ecoinvent	Grobbelaar, 2004
Mono calcium phosphate	75.5	kg/t algal biomass	replaced by single superphoshate (21% of P_2O_5)	RER: single superphosphate, as P_2O_5, at regional storehouse	Ecoinvent	Grobbelaar, 2004
	WASTEWATER CULTIVATION					
Water	1424.5	m^3/t algal biomass	from wastwater treatment	Water (wastewater, untreated) [Production residues in life cycle]	Ecoinvent	Brentner et al., 2011
Nitrogen	234.3	kg/t algal biomass	content in wastewater and without added	Nitrogen (N-compounds) [Inorganic emissions to air]	Ecoinvent	Brentner et al., 2011
Phosphorus	75.5	kg/t algal biomass	content in wastewater and without added	Phosphorus [Inorganic emissions to air]	Ecoinvent	Brentner et al., 2011

Table 4.1: inventory for cultivation, assuming 1 ton of wet algal biomass

4.1.2 Data for harvesting and drying

For harvesting, aluminum or lime flocculation and centrifugation are alternatively considered. In centrifugation, only electricity is used while in flocculation, the use of flocculants is coupled with electricity use. Input for harvesting and drying are illustrated in Table 4.2. In drying phase, heat is provided when hexane extraction is performed.



		HARVESTING				
		FLOCCULATION				
Flow	Amount	Unit	Comment	Process in Gabi	Database	Source of reference
Algal Biomass	1	kg				
Electricity Use						
Electricity consumption in flocculation	149.9	kWh/t algal biomass		DK: Electricity production mix	Ecoinvent	Brentner et al., 2011
Flocculant						
Aluminium	105	kg/ t algal biomass		RER: aluminium sulphate powder at plant	Ecoinvent	Grima et al., 2003
Lime	449	kg/ t algal biomass		CH: lime hydrated packed at plant	Ecoinvent	Lardon et al., 2009
		CENTRIFUGATION				
Electricity consumption in centrifugation	1449.5	kWh/t algal biomass		DK: Electricity production mix	Ecoinvent	Foley et al., 2011
		DRYING				
Algal Biomass	3.45	kg of wet biomass	Input flow: water content 71%			Singh, 2012
Dry biomass	1	kg	Assuming that which it is not water it is lipid content			Singh, 2012
Heat	6	MJ/kg of algal biomass	Only when hexane extraction is carried out	RER: heat, unspecific at chemical plant	Ecoinvent	Xu et al., 2011

Table 4.2: inventory for harvesting and drying. Drying is performed only if hexane extraction is carried out. When supercritical CO_2 extraction is considered, drying is avoided, as described in section 3.2.5

4.1.3 Data for algal oil extraction and transesterification

Inputs for algal oil extraction and for transesterification are described in the Table 4.3 and in the Table 4.4.

		EXTRACTION				
		EXTRACTION WITH HEXANE				
Flow	Amount	Unit	Comment	Process in Gabi	Database	Source of reference
Dry biomass	1	kg	input flow			Singh, 2012
Extraction efficiency	0.91		assuming lipid content as 29% of dry biomass			Singh, 2012
Electricity Use						
Electricity consumption in hexane extraction	232.18	kWh/t biodiesel		DK: electricity production mix	Ecoinvent	Brentner et al., 2011
Heat						
Heat	3935.19	MJ/ t biodiesel		RER: heat unspecific at plant	Ecoinvent	Brentner et al., 2011
Hexane	15.2	kg/t biodiesel		RER: hexane at plant	Ecoinvent	Lardon et al., 2009
		SUPERCRITICAL CO2 EXTRACTION				
CO_2 liquid	145	liter	assuming 27.5 MPa and 47.5°C as condition of supercritical extarction	RER: carbon dioxide liquid at plant	Ecoinvent	Mendes et al., 1995
Electricity Use						
Electricity	7201.39	kWh/t biodiesel		DK: electricity production mix	Ecoinvent	Singh, 2012

Table 4.3: inventory for algal oil extraction

		TRANSESTERIFICATION				
Flow	Amount	Unit	Comment	Process in Gabi	Database	Source of reference
Algal oil	1	kg				Singh et al., 2012
Conversion efficiency	98%					Brentner et al., 2011
Electricity Use						
Total Electricity consumption in transesterification	39.35	kWh/t biodiesel		DK: Electricity production mix	Ecoinvent	Brentner et al., 2011
Heat						
Heat	885.42	MJ/ t biodiesel		RER: Heat unspecific at plant	Ecoinvent	Brentner et al., 2011
Methanol	114	kg/biodiesel		GLO: methanol at plant	Ecoinvent	Lardon et al., 2009

Table 4.4: inventory for transesterification

4.1.4 Data for system expansion: anaerobic digestion and glycerol use

In this section, Table 4.5, Table 4.6 and Table 4.7 illustrate data for system expansion. Input for biogas, electricity in anaerobic digestion and propylene glycol production in use of glycerol are described as follows.

		ANAEROBIC DIGESTION				
		PRODUCTION OF BIOGAS				
Flow	Amount	Unit	Comment	Process in Gabi	Database	Source of reference
Electricity	0.2626	MJ		CH:electricity, low voltage, at grid	Ecoinvent	GaBi 4
Plant for Anaerobic digestion	7.30E-09	pcs		CH: anaerobic digestion plant, biowaste	Ecoinvent	GaBi 4
Transport	0.018	tkm		CH: transport, lorry 20-28t, fleet average	Ecoinvent	GaBi 4
Transport for municipal waste	0.01597	tkm		CH: transport, municipal waste collection, lorry 21t	Ecoinvent	GaBi 4
Heat	1.08	MJ		RER: heat, natural gas, at boiler condensing modulating >100kW	Ecoinvent	GaBi 4
Municipal solid waste	0.0159	kg	Assuming 0% of water. It also is assumed that municipal solid waste is as residual biomass from extraction	CH: disposal, municipal solid	Ecoinvent	GaBi 4
		OUTPUT				
Biogas from biowaste	1	Nm^3		CH: biogas, from biowaste, at storage [fuels]	Ecoinvent	GaBi 4

Table 4.5: inventory of biogas production

		ELECTRICITY FROM BIOGAS				
Lubricating oil	0.00026118	kg		RER: lubricating oil, at plant	Ecoinvent	GaBi 4
Cogen unit for electricity	5.63E-08	pcs		RER: cogen unit 160kWe, components for electricity only	Ecoinvent	GaBi 4
Disposal of oil	0.00026118	kg		CH: disposal, used mineral oil, 10% water, to hazardous waste incineration	Ecoinvent	GaBi 4
Cogen unit for electricity and heat	4.35E-08	pcs		RER: cogen unit 160kWe, common components for heat+electricity	Ecoinvent	GaBi 4
Biogas	0.38298	Nm^3		CH: biogas, production mix, at storage [fuels]	Ecoinvent	GaBi 4

Table 4.6: inventory of electricity (1 kWh) production from biogas

		USE OF GLYCERINE TO PRODUCE PROPYLENE GLYCOL				
Flow	Amount	Unit	Comment	Process in Gabi	Database	Source of reference
Glycerol	0.803	kg/kg of propylene glycol	assuming glycerol conversion as 0.02		Ecoinvent	Ecoinvent
Electricity Use						
Electricity use in propylene glycol production	1.20	MJ/ kg of propylene glycol		UCTE: electricity, medium voltage, production UCTE, at grid [production mix]	Ecoinvent	Ecoinvent
Heat						
Heat	2	MJ/ kg of propylene glycol		RER: heat, natural gas, at industrial furnace >100kW	Ecoinvent	Ecoinvent
Others						
Transport in street	0.0803	tkm		RER: transport, lorry >16t, fleet average [Street]	Ecoinvent	Ecoinvent
Transport in railway	0.482	tkm		RER: transport, freight, rail [Railway]	Ecoinvent	Ecoinvent
Chemical plant	4.00E-10	pcs./kg of propylene glycol		RER: chemical plant, organics	Ecoinvent	Ecoinvent

Table 4.7: inventory for propylene glycol production from algal glycerol

4.2 Modeling the system with Gabi 4.0

The system was modeled with Gabi 4.0. GaBi is developed by PE international and it analyzes product life cycles or process technologies. GaBi models each element of a product or system from a life-cycle perspective and it is used to make the best informed decisions on the manufacture and lifecycle of any product. It is also possible creating processes and plans used and adapted for the model.

In this software, the database used is Ecoinvent, in which processes and flows are considered. Due to Ecoinvent is a Swiss database, some of these processes take place in Switzerland. Many processes refer to European country (RER).

All flows are calculated quantifying environmental impacts, splitted in each impact category. It is also possible to choice the method for impact assessment.

4.2.1 Assumptions in GaBi model

Since some of materials and processes are missing, the materials and processes used are not too much accurate; hence those are replaced by other similar, which could lead to inaccuracy in the emission estimation.

Most of the different processes used are from Europe, since the real location is not available in GaBi. European processes are used in order to model a consistent biodiesel process with the geographical scope. Some assumptions are made.

Nannochloropsis is cultivated, assuming its lipid content as 29% (Rodolfi et al., 2009) of dry biomass and the remaining part of the dry biomass is assumed to be proteins (30%), carbohydrates (10%) and other compounds (31%) (Razon and Tan, 2011).

Ammonium nitrate contains 35% of nitrogen.

Due to the same contents of PO_4^{3-} (21%), monocalcium phosphate is replaced by single superphosphate.

In the harvesting, the main flocculants used are aluminum sulphate and lime.

The extraction efficiency of algal oil is assumed to be 91% and the conversion efficiency for biodiesel is 98%. Hence, the glycerol conversion efficiency is 2%. It is also assumed that glycerin from soybean oil is equal to glycerol form algal oil.

The amount of hexane and methanol are estimated by Lardon and coauthors (2009).

Electricity production mix from Denmark is used for cultivation, harvesting, extraction and transesterification. The composition of Danish electricity mix is shown in Figure 4.1.

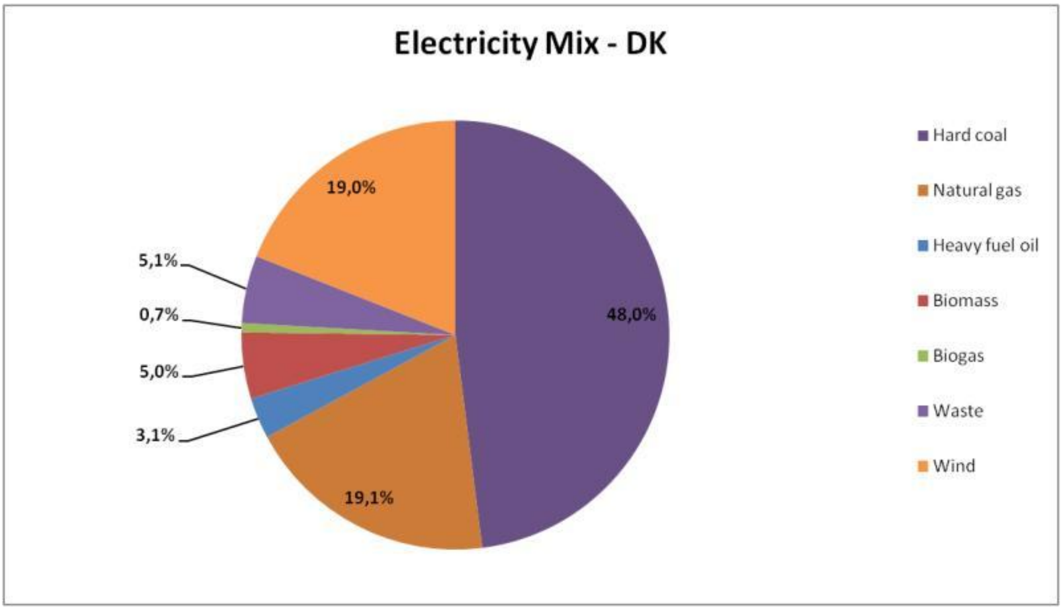


Figure 4.1: Danish electricity production mix (PE INTERNATIONAL, GaBi database, 2008)

Hard coal (48%), natural gas and wind (19%) are the most important electricity sources for Denmark.

Since the heating used in biodiesel production system is not specified in the inventory data, heat unspecific in chemical plant is chosen as the main representative for the system.

Due to residual biomass is used after its drying, in anaerobic digestion water content is assumed to be 0%.

Nevertheless these assumptions could lead to uncertainties in the analysis; the estimations will be reviewed in order to reduce the uncertainties as limited as possible.

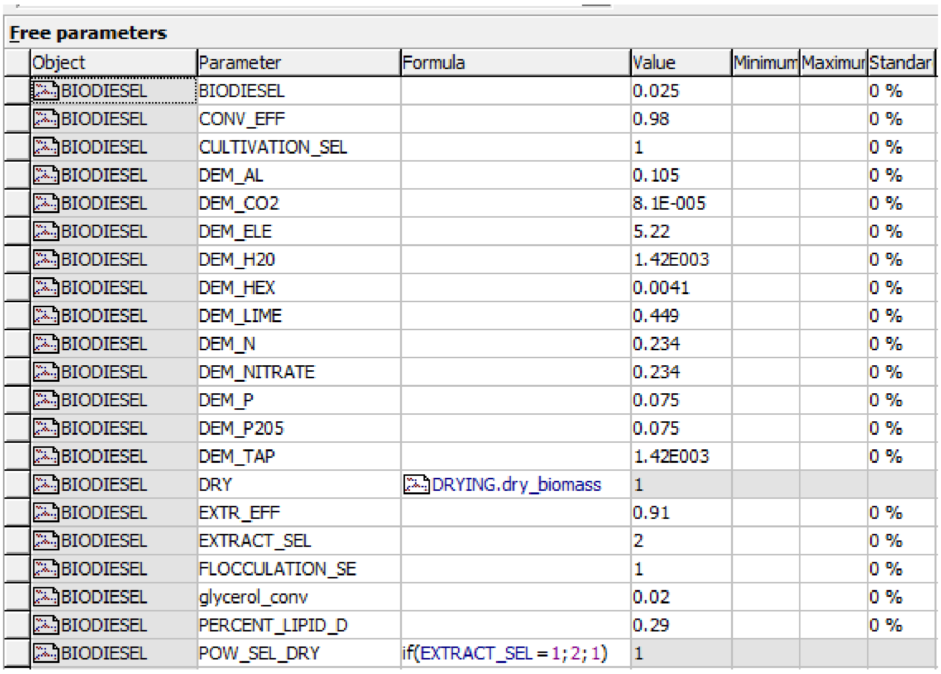
4.2.2 Model of biodiesel production system

Since functional unit and reference flow are 1 MJ of biodiesel (calorific value is 39.35 MJ/kg of biodiesel), all flows will be calculated respect to 0.025 kg of biodiesel in GaBi model. Each input is referred to functional unit. For this reason, TRANSESTERIFICATION is the fixed process. This setting implies that all inputs are scaled for functional unit.

The model of the system is based on a top plan “BIODIESEL”, in which main processes are considered like a sub plan (Figure 4.4). BIODIESEL plan is shown in the Figure 4.3.

BIODIESEL plan is parameterized at the top level. If parameters change in the global level, they have to change in their respective sub plans, as well.

In order to know which parameters are considered in BIODIESEL plan, it is possible to use parameter explorer and all free parameters are shown in Figure 4.2. In order to make a sensitivity analysis, some parameters could be the most relevant in the biodiesel production process. These parameters are extraction efficiency (extract_eff) and lipid ratio in dry biomass (percent_lipid_d). Other important parameters are conversion efficiency (conv_eff) and glycerol conversion (glycerol_conv). Due to a modeling of different scenarios, water for cultivation, flocculation and extraction selection (cultivation_sel, harvesting_sel and extraction_sel) allow the choice of different options used for each process.



Free parameters

Object	Parameter	Formula	Value	Minimum	Maximum	Standar
BIODIESEL	BIODIESEL		0.025			0 %
BIODIESEL	CONV_EFF		0.98			0 %
BIODIESEL	CULTIVATION_SEL		1			0 %
BIODIESEL	DEM_AL		0.105			0 %
BIODIESEL	DEM_CO2		8.1E-005			0 %
BIODIESEL	DEM_ELE		5.22			0 %
BIODIESEL	DEM_H20		1.42E003			0 %
BIODIESEL	DEM_HEX		0.0041			0 %
BIODIESEL	DEM_LIME		0.449			0 %
BIODIESEL	DEM_N		0.234			0 %
BIODIESEL	DEM_NITRATE		0.234			0 %
BIODIESEL	DEM_P		0.075			0 %
BIODIESEL	DEM_P205		0.075			0 %
BIODIESEL	DEM_TAP		1.42E003			0 %
BIODIESEL	DRY	DRYING.dry_biomass	1			
BIODIESEL	EXTR_EFF		0.91			0 %
BIODIESEL	EXTRACT_SEL		2			0 %
BIODIESEL	FLOCCULATION_SE		1			0 %
BIODIESEL	glycerol_conv		0.02			0 %
BIODIESEL	PERCENT_LIPID_D		0.29			0 %
BIODIESEL	POW_SEL_DRY	if(EXTRACT_SEL = 1; 2; 1)	1			

Figure 4.2: GaBi parameter explorer. All free parameters are shown. In biodiesel production, most relevant parameters are conv_eff, glycerol_eff, extr_eff and percent_lipid. In order to model different scenario, cultivation_sel, extract_sel and flocculation_sel allow the choice of different options. These options are described in section 3.2.5

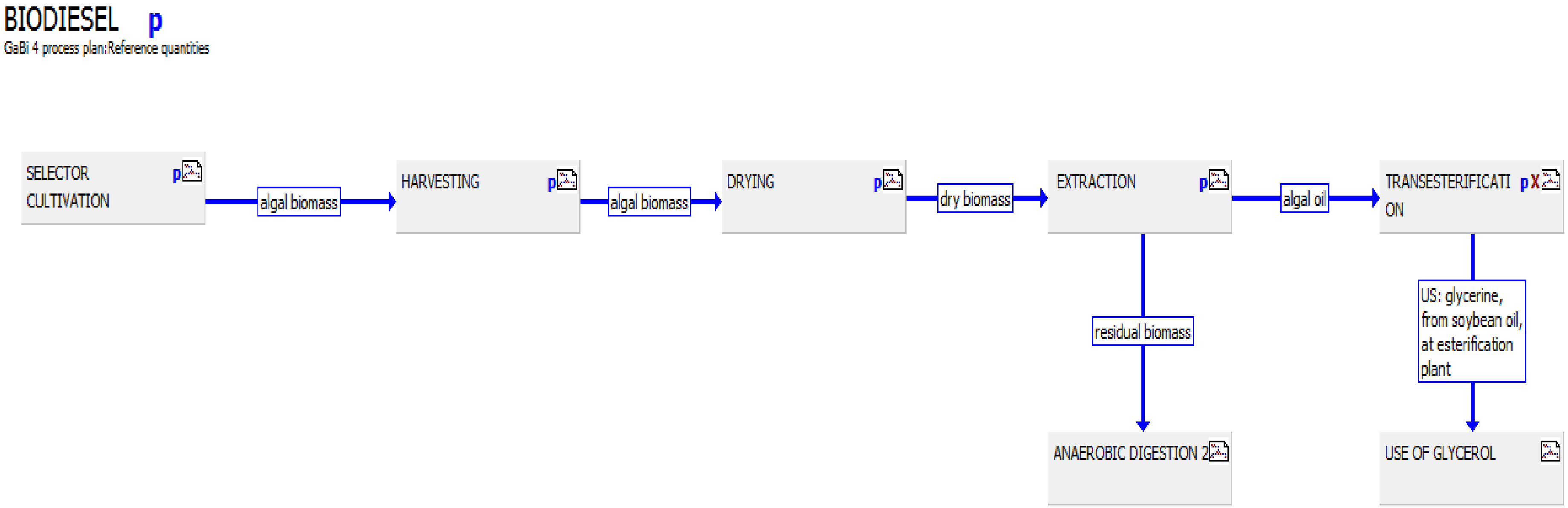


Figure 4.3: modelling of biodiesel production in Denmark with GaBi 4.0

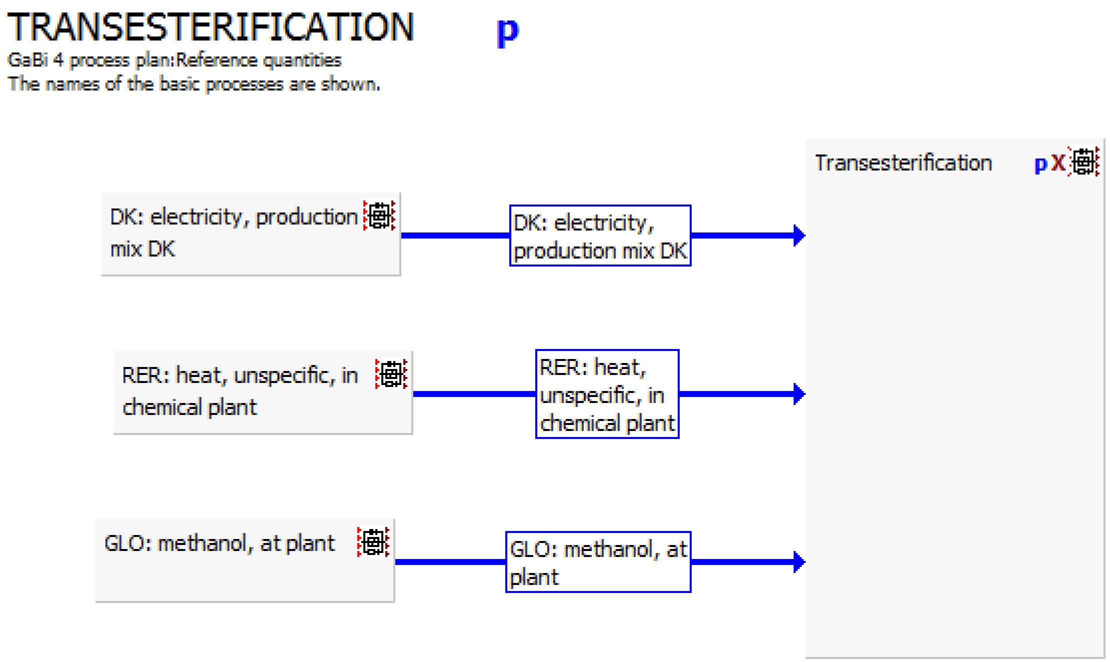


Figure 4.4: an example of sub plan

5 LIFE CYCLE IMPACT ASSESSMENT

LCI results have been quantified in environmental impacts by LCIA.

In section 5.1, for each impact category, all 24 scenarios are compared to diesel production, considering both synthetic CO_2 and waste CO_2. In sections 5.2 and 5.3, the analysis of contributions of different phases to environmental impacts is limited to the scenarios 1 and 4, since lime flocculation and centrifugation show impacts in the same order of magnitude than those of aluminum flocculation. In these sections, results are discussed as follows. Firstly, contributions of each phase to the different impact categories are described for the basic scenario “aluminum flocculation and hexane extraction” (scenario 1) as well as contributions of each process. This means an analysis of different options in terms of environmental impacts. Results of cultivation both in wastewater and freshwater with both synthetic CO_2 and waste CO_2 are described. Additionally, scenario “aluminum flocculation and sCO_2 extraction” (scenario 4) is compared with scenario 1 in order to highlight the main differences between hexane and sCO_2 extraction for each impact category.

In the appendix 9.1, scenarios 2, 3, 5 and 6 are described in terms of relative contribution to each impact category.

5.1 Classification and characterization

In this section, LCIA results are shown.

Table 5.1 and Table 5.2 show the results of the 24 scenarios for all impact categories. Table 5.1 analyzes different scenarios considering the use of synthetic CO_2 while Table 5.2 investigates scenarios when waste CO_2 is used. A comparison between diesel and algal biodiesel is shown in both of these tables. Impacts higher than those of diesel are in red while the lower ones are in green.

Impact categories (synthetic CO_2)	diesel	fresh al hex	fresh li hex	fresh centr hex	fresh al co_2	fresh li co_2	fresh centr co_2	waste al hex	waste li hex	waste centr hex	waste al co_2	waste li co_2	waste centr co_2
Aquatic acidification [kg SO2-Eq. to air]	9.73E-05	1.78E-02	1.72E-02	1.92E-02	1.54E-02	1.48E-02	1.69E-02	1.00E-02	9.39E-03	1.15E-02	7.67E-03	7.02E-03	9.10E-03
Aquatic eutrophication [kg PO4-Eq. to water]	3.39E-07	4.34E-04	4.22E-04	4.25E-04	4.28E-04	4.16E-04	4.19E-04	-2.22E-01	-2.22E-01	-2.22E-01	-2.22E-01	-2.22E-01	-2.22E-01
Carcinogens [kg C2H3Cl-Eq. to air]	1.24E-05	8.63E-03	8.29E-03	8.46E-03	7.89E-03	7.55E-03	7.72E-03	4.03E-03	3.70E-03	3.86E-03	3.29E-03	2.96E-03	3.12E-03
Global warming 500yr [kg CO2-Eq. to air]	1.06E-02	5.95E+00	6.23E+00	6.71E+00	4.60E+00	4.88E+00	5.37E+00	4.01E+00	4.29E+00	4.78E+00	2.66E+00	2.94E+00	3.43E+00
Non-carcinogens [kg C2H3Cl-Eq. to air]	1.91E-04	8.27E-02	7.85E-02	8.00E-02	7.49E-02	7.06E-02	7.21E-02	3.43E-02	3.00E-02	3.15E-02	2.64E-02	2.22E-02	2.34E-02
Non-renewable energy consumption [MJ]	1.21E+00	8.27E+01	8.36E+01	9.27E+01	6.26E+01	6.36E+01	7.26E+01	5.93E+01	6.03E+01	6.94E+01	3.93E+01	4.02E+01	4.83E+01
Ozone layer depletion [kg CFC-11-Eq. to air]	1.03E-08	4.86E-07	5.05E-07	5.26E-07	3.23E-07	3.42E-07	3.63E-07	3.43E-07	3.62E-07	3.83E-07	1.80E-07	1.99E-07	2.17E-07
Photochemical oxidation [kg C2H4-Eq. to air]	1.82E-05	1.11E-03	1.18E-03	1.20E-03	8.67E-04	9.30E-04	9.50E-04	7.78E-04	8.42E-04	8.61E-04	5.31E-04	5.94E-04	6.05E-04
Terrestrial acidification [kg SO2-Eq. to air]	3.18E-04	9.42E-02	9.40E-02	1.01E-01	8.37E-02	8.35E-02	9.06E-02	3.94E-02	3.92E-02	4.62E-02	2.89E-02	2.87E-02	3.58E-02

Table 5.1: impact categories for each scenario considering synthetic CO_2

Impact categories (waste CO_2)	diesel	fresh al hex	fresh li hex	fresh centr hex	fresh al co2	fresh li co2	fresh centr co2	waste al hex	waste li hex	waste centr hex	waste al co2	waste li co2	waste centr co2
Aquatic acidification [kg SO2-Eq. to air]	9.73E-05	1.53E-02	1.46E-02	1.67E-02	1.29E-02	1.22E-02	1.43E-02	7.50E-03	6.85E-03	8.93E-03	5.13E-03	4.48E-03	6.56E-03
Aquatic eutrophication [kg PO4-Eq. to water]	3.39E-07	4.01E-04	3.89E-04	3.92E-04	3.95E-04	3.83E-04	3.86E-04	-2.22E-01	-2.22E-01	-2.22E-01	-2.22E-01	-2.22E-01	-2.22E-01
Carcinogens [kg C2H3Cl-Eq. to air]	1.24E-05	6.28E-03	5.95E-03	6.12E-03	5.55E-03	5.21E-03	5.38E-03	1.69E-03	1.36E-03	1.52E-03	9.50E-04	6.18E-04	7.81E-04
Global warming 500yr [kg CO2-Eq. to air]	1.06E-02	3.11E+00	3.39E+00	3.88E+00	1.77E+00	2.05E+00	2.54E+00	1.18E+00	1.46E+00	1.94E+00	-1.67E-01	1.13E-01	5.99E-01
Non-carcinogens [kg C2H3Cl-Eq. to air]	1.91E-04	6.59E-02	6.17E-02	6.32E-02	5.81E-02	5.38E-02	5.53E-02	1.75E-02	1.32E-02	1.47E-02	9.58E-03	5.35E-03	6.81E-03
Non-renewable energy consumption [MJ]	1.21E+00	6.51E+01	6.60E+01	7.51E+01	4.50E+01	4.60E+01	5.51E+01	4.18E+01	4.27E+01	5.18E+01	2.17E+01	2.26E+01	3.17E+01
Ozone layer depletion [kg CFC-11-Eq. to air]	1.03E-08	3.91E-07	4.10E-07	4.32E-07	2.29E-07	2.48E-07	2.69E-07	2.49E-07	2.67E-07	2.89E-07	8.59E-08	1.05E-07	1.26E-07
Photochemical oxidation [kg C2H4-Eq. to air]	1.82E-05	8.14E-04	8.78E-04	8.97E-04	5.67E-04	6.30E-04	6.50E-04	4.78E-04	5.41E-04	5.61E-04	2.30E-04	2.94E-04	3.13E-04
Terrestrial acidification [kg SO2-Eq. to air]	3.18E-04	8.35E-02	8.33E-02	9.03E-02	7.30E-02	7.28E-02	7.99E-02	2.86E-02	2.85E-02	3.55E-02	1.82E-02	1.80E-02	2.51E-02

Table 5.2: impact categories for each scenario considering waste CO_2

5.1.1 Global warming

Figure 5.1 shows the results of the impact on the global warming (in kg of CO_2-eq.) obtained assuming the use of synthetic CO_2 during the phase of cultivation. Results show that scenarios using wastewater have GWPs lower than the ones using the freshwater. As it is possible to observe from the figure, GWPs for algal biodiesel production are higher than those for diesel production in both “freshwater” and “wastewater scenarios” by about two order of magnitude.

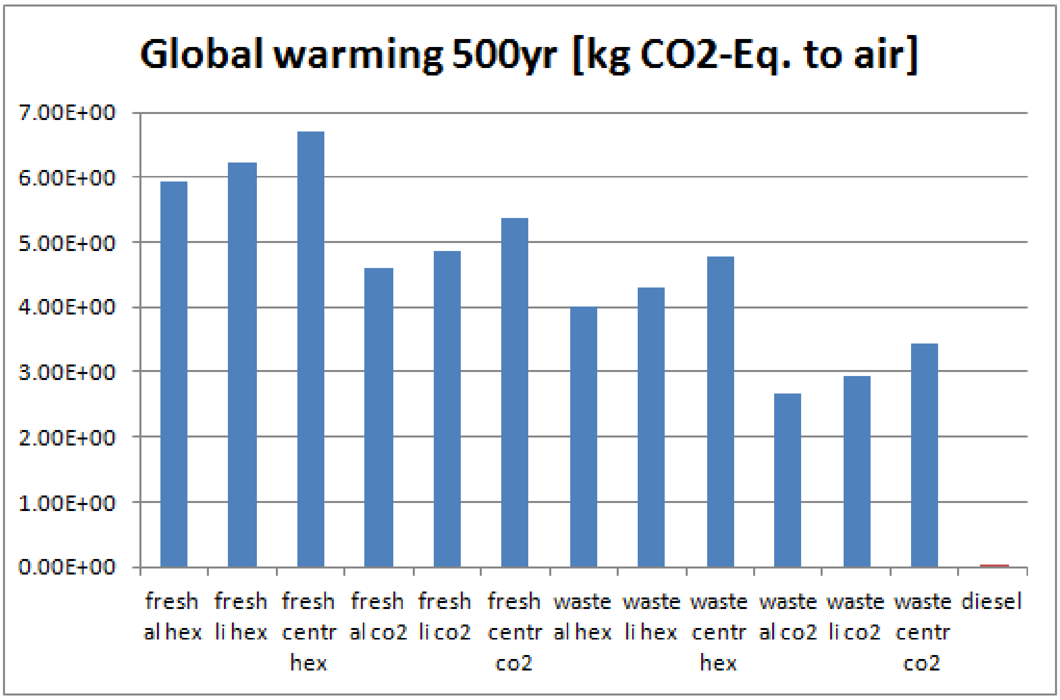


Figure 5.1: GWP (500 years) when synthetic CO_2 is used. GWP for algal biodiesel is compared to diesel

Figure 5.2 shows the results of the impact on the global warming (in kg of CO_2-eq) obtained assuming the use of waste CO_2 during the phase of cultivation. Also in this case, GWPs for algal biodiesel production are higher than those for diesel, except for the scenario “aluminum flocculation and sCO_2 extraction”. In addition, the scenario assuming extraction with sCO_2 has a negative GWP, indicating avoided emissions of GHG in atmosphere. This interesting result highlights that the waste CO_2 use can decrease GHG emissions, in fact the use of a waste flow does not take into account impacts related to its production. In order to implement a large scale production, sCO_2 extraction could be an interesting option due to the fact that drying phase is avoided and it does require moderate high pressures (20-30 MPa) and temperatures (25°C-30°C), as stated by Herrero and coauthors (2006). On the other hand, cultivation in wastewater using waste CO_2 still needs to be improved on a commercial scale.

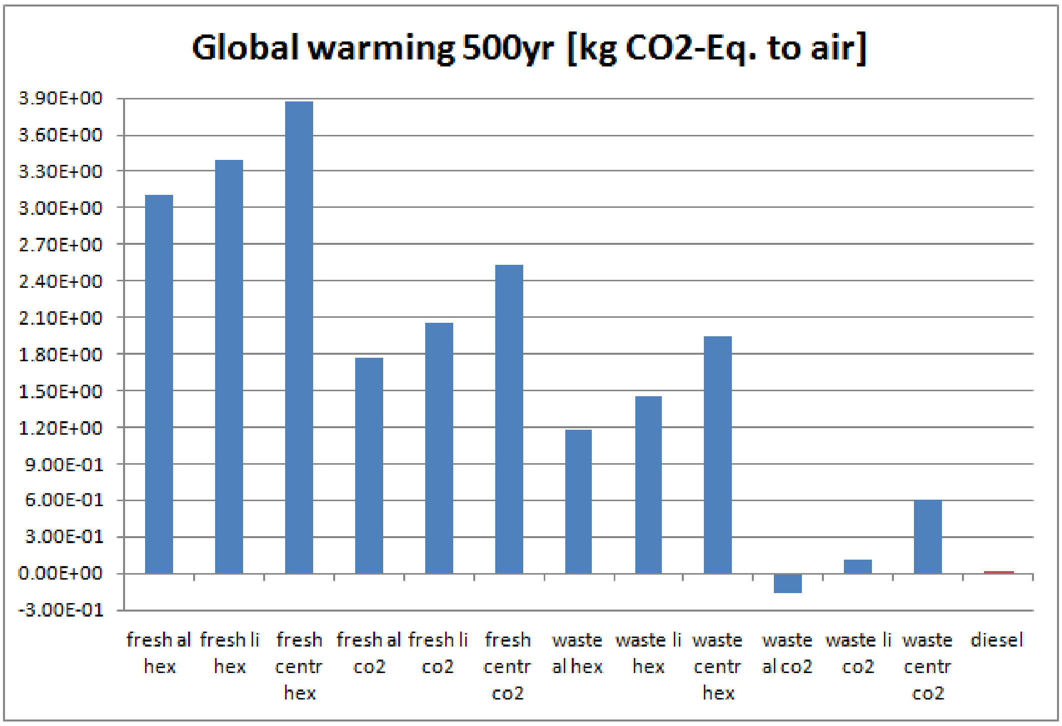


Figure 5.2: GWP when waste CO_2 is used. GWPs for algal biodiesel is compared to diesel

5.1.2 Non renewable energy consumption

Non renewable energy consumption is shown in Figure 5.3 and in Figure 5.4 when synthetic and waste CO_2 are alternatively considered. In both case, the difference between diesel and algal biodiesel is about one order of magnitude in all scenarios. This means that the biodiesel production requires more energy than that is produced by 1 MJ of biodiesel.

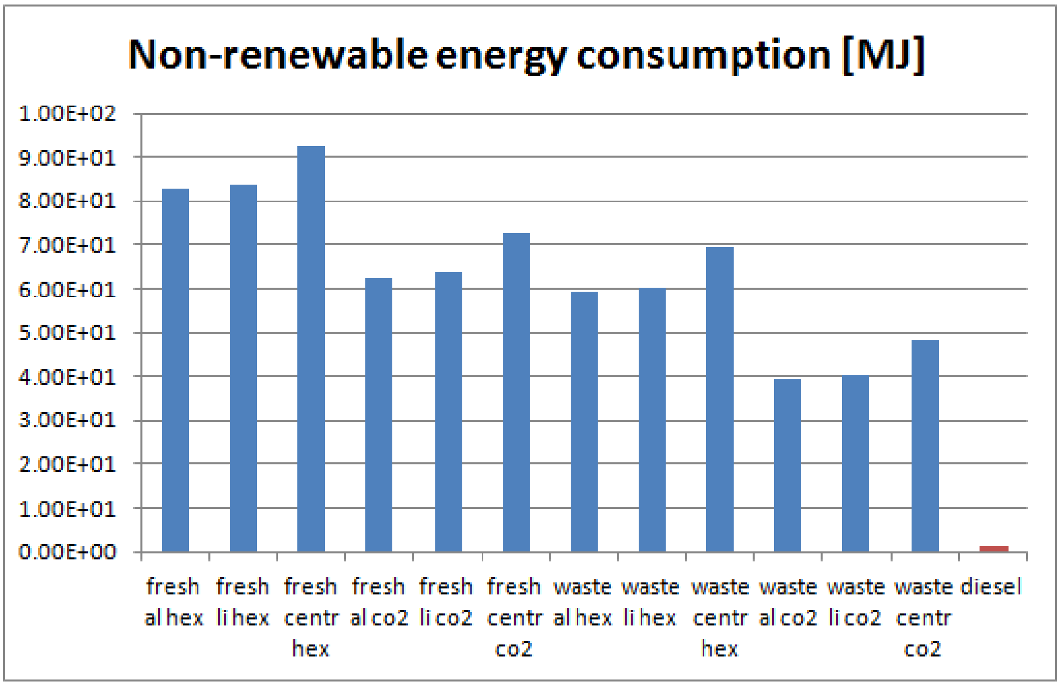


Figure 5.3: non renewable energy consumption for each scenario using synthetic CO_2. Comparison between algal biodiesel and diesel

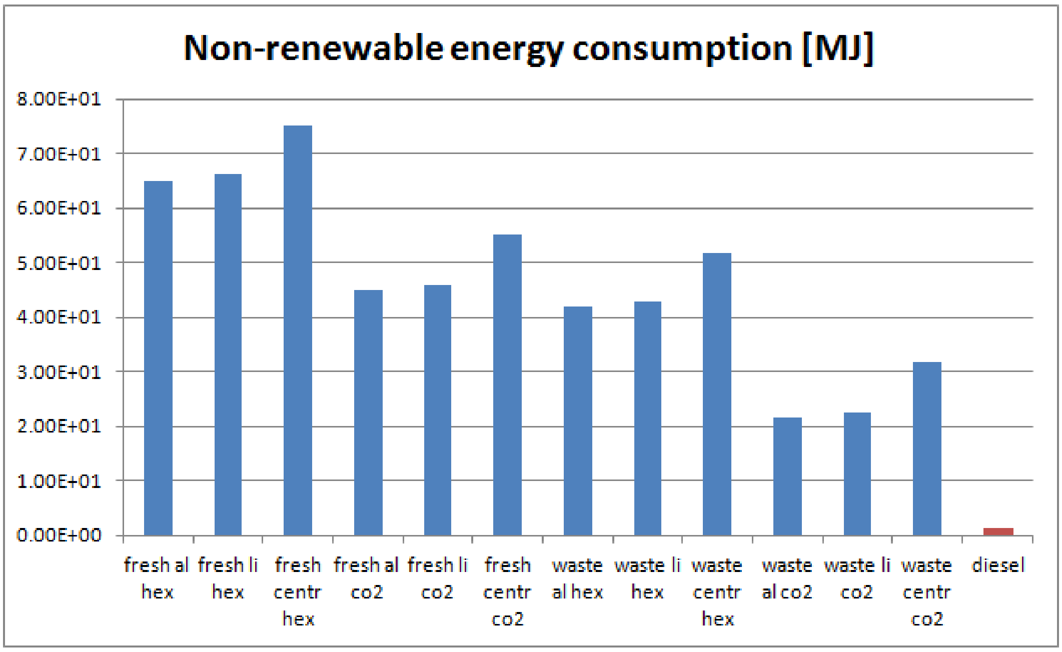


Figure 5.4: non renewable energy consumption for each scenario using waste CO_2. Comparison between algal biodiesel and diesel

5.1.3 Aquatic eutrophication

Table 5.3 summarizes EP for each scenario. Since ammonium nitrate and single superphosphate are added to water in freshwater scenarios, EP for algal biodiesel is higher than diesel in both synthetic CO_2 and waste CO_2. On the other hand, the use of wastewater avoids impacts because fertilizers are not necessary. This is due to the nitrogen and phosphorus availability in wastewater (see section 1.2.4). Difference between "freshwater and wastewater scenarios" corresponds to three order of magnitude and negative values for "wastewater scenarios" mean avoided impacts.

Aquatic eutrophication [kg PO4-Eq. to water]	fresh al hex	fresh li hex	fresh centr hex	fresh al CO_2	fresh li CO_2	fresh centr CO_2	waste al hex	waste li hex	waste centr hex	waste al CO_2	waste li CO_2	waste centr CO_2	diesel
Synthetic CO_2	4.34E-04	4.22E-04	4.25E-04	4.28E-04	4.16E-04	4.19E-04	-2.22E-01	-2.22E-01	-2.22E-01	-2.22E-01	-2.22E-01	-2.22E-01	3.39E-07
Waste CO_2	4.01E-04	3.89E-04	3.92E-04	3.95E-04	3.83E-04	3.86E-04	-2.22E-01	-2.22E-01	-2.22E-01	-2.22E-01	-2.22E-01	-2.22E-01	3.39E-07

Table 5.3: contributions to eutrophication for all scenarios, considering synthetic CO_2 and waste CO_2, respectively.

5.1.4 Ozone depletion

ODPs are shown in Figure 5.5 and in Figure 5.6. ODPs for algal biodiesel production are higher than diesel production in both cases for all scenarios. The difference between the use of waste and synthetic CO_2 is not relevant and the ODP values are in the same order of magnitude. Obviously, "wastewater scenarios" show lower ODPs than those of "freshwater scenarios".

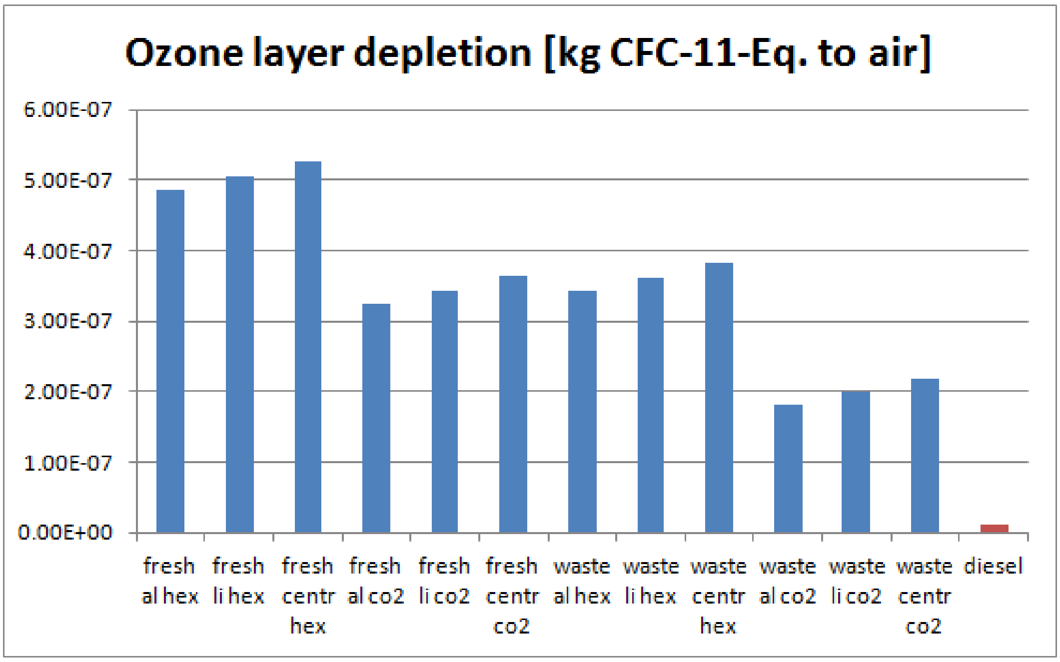


Figure 5.5: ODPs for algal biodiesel using synthetic CO_2

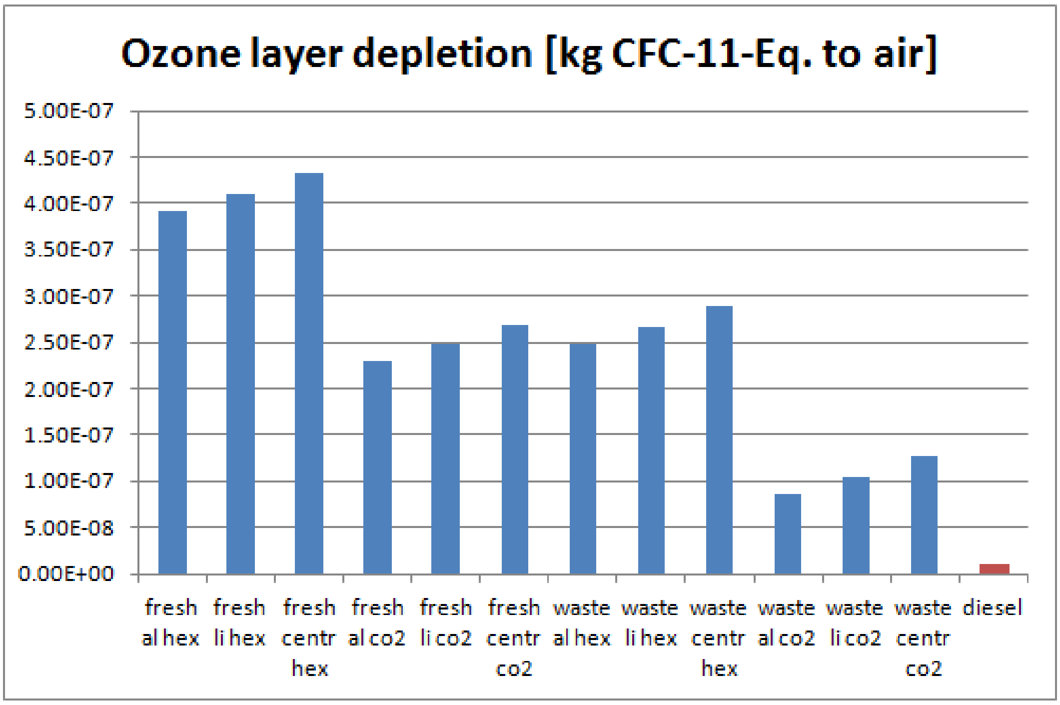


Figure 5.6: ODPs for algal biodiesel using waste CO_2

5.1.5 Photochemical oxidation

POCP is illustrated in Figure 5.7 and in Figure 5.8. All scenarios are worse than diesel when both synthetic CO_2 and waste CO_2 are used. Table 5.1 and Table 5.2 display POCP values for all scenarios. Differences between synthetic and waste CO_2 are not relevant, in fact all scenarios are in the same order of magnitude.

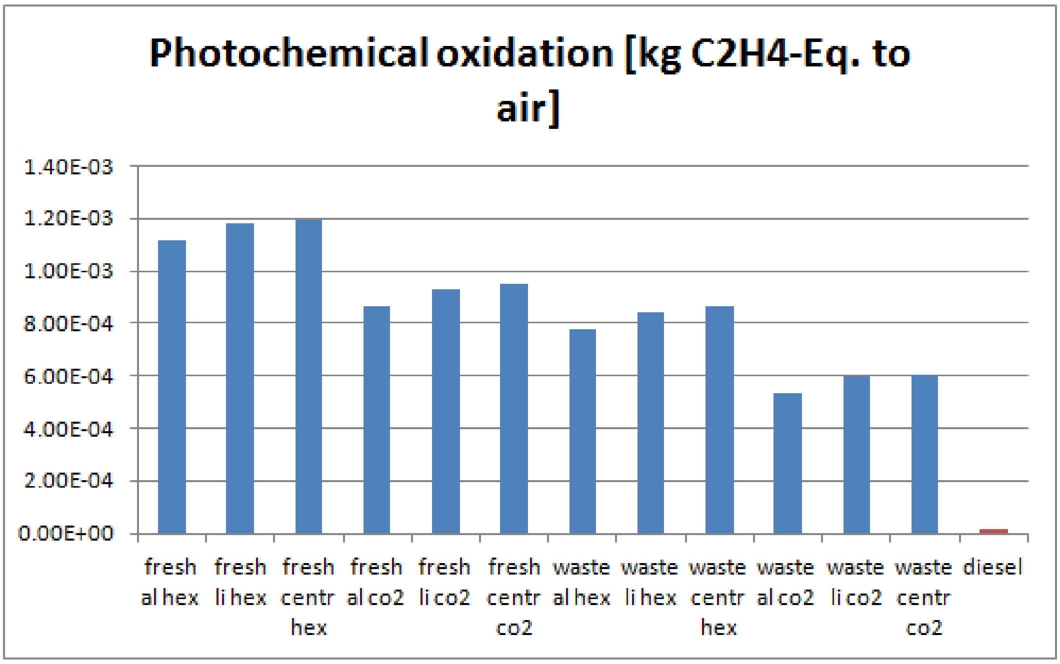


Figure 5.7: POCP for algal biodiesel is compared to diesel. All scenarios are performed with the use of synthetic CO_2

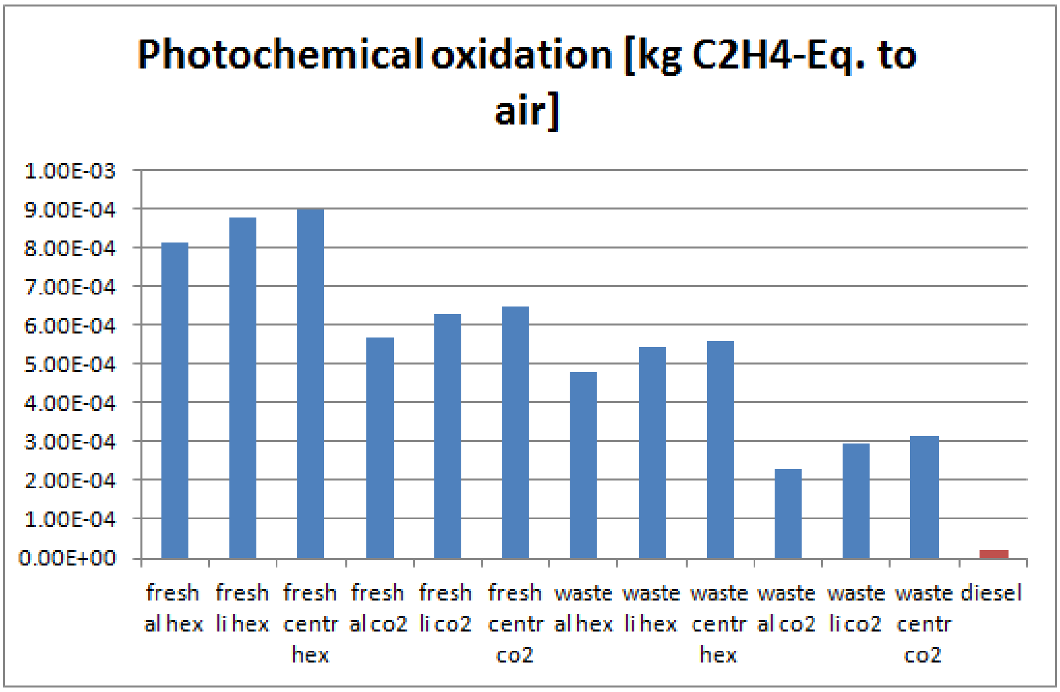


Figure 5.8: POCP for algal biodiesel is compared to diesel. All scenarios are performed with the use of waste CO_2

5.1.6 Acidification

5.1.6.1 Aquatic acidification

Figure 5.9 and Figure 5.10 show aquatic AP considering the use of synthetic and waste CO_2 respectively. Compared to diesel, all 24 scenarios have higher impacts for aquatic AP by about one order of magnitude.

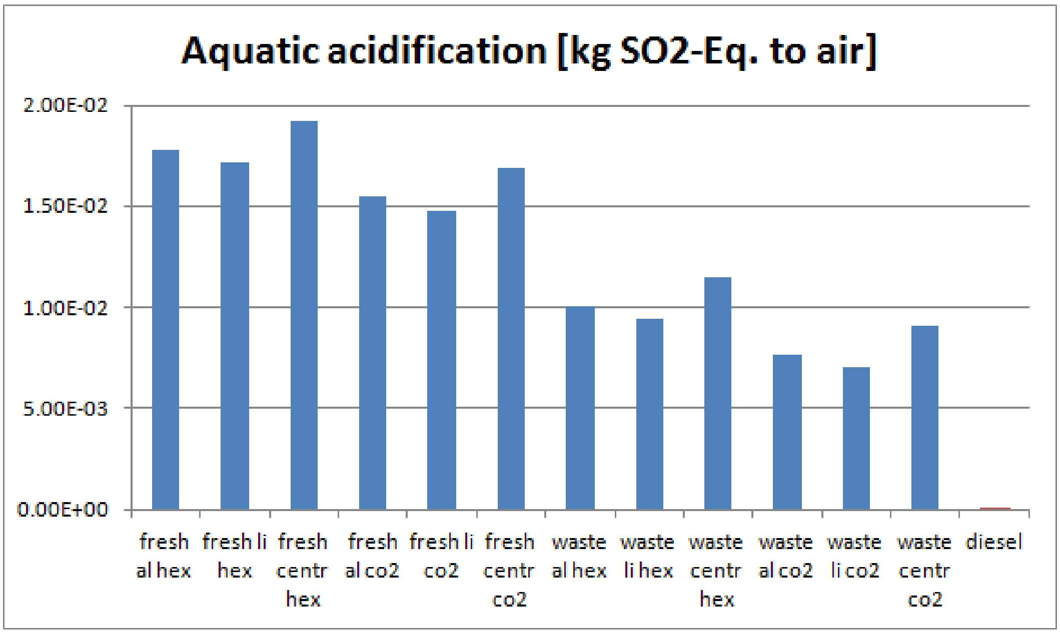


Figure 5.9: aquatic AP for algal biodiesel is compared to diesel. These scenario are performed with the use of synthetic CO_2

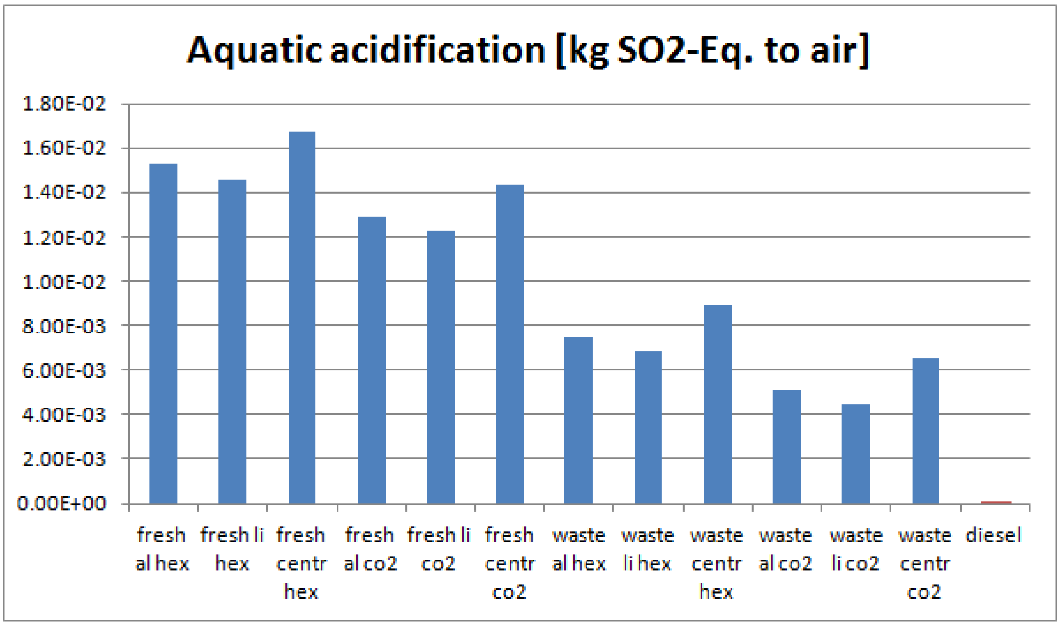


Figure 5.10: aquatic acidification for algal biodiesel is compared to diesel. These scenario are performed with the use of waste CO_2

5.1.6.2 Terrestrial acidification

Terrestrial AP is illustrated in Figure 5.11 and in Figure 5.12 using synthetic and waste CO_2.

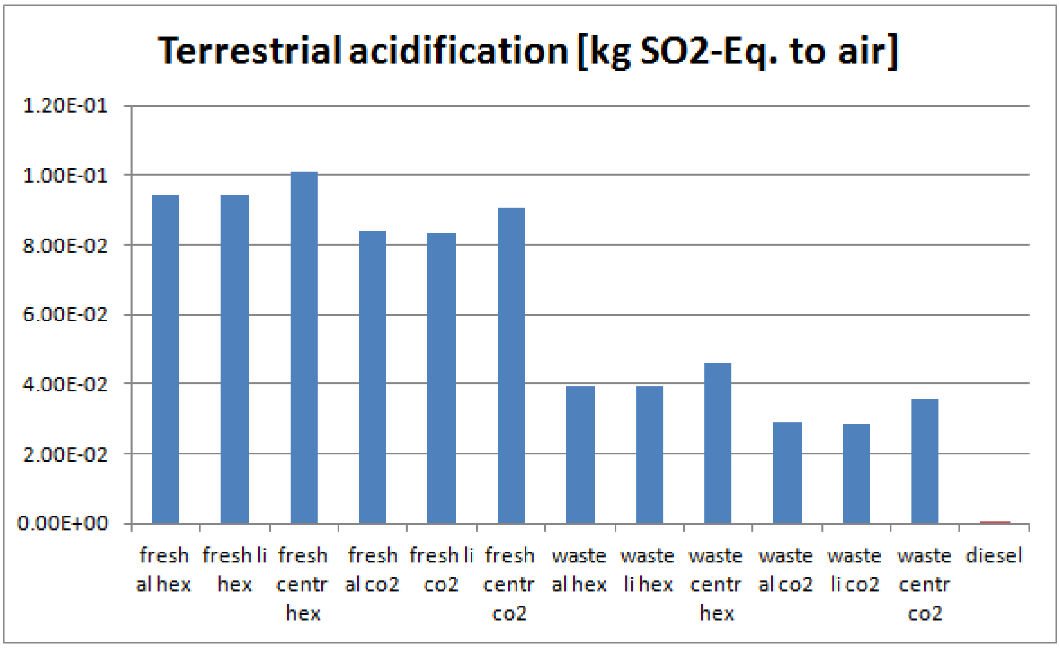


Figure 5.11: terrestrial AP for algal biodiesel is compared to diesel. These scenario are performed with the use of synthetic CO_2

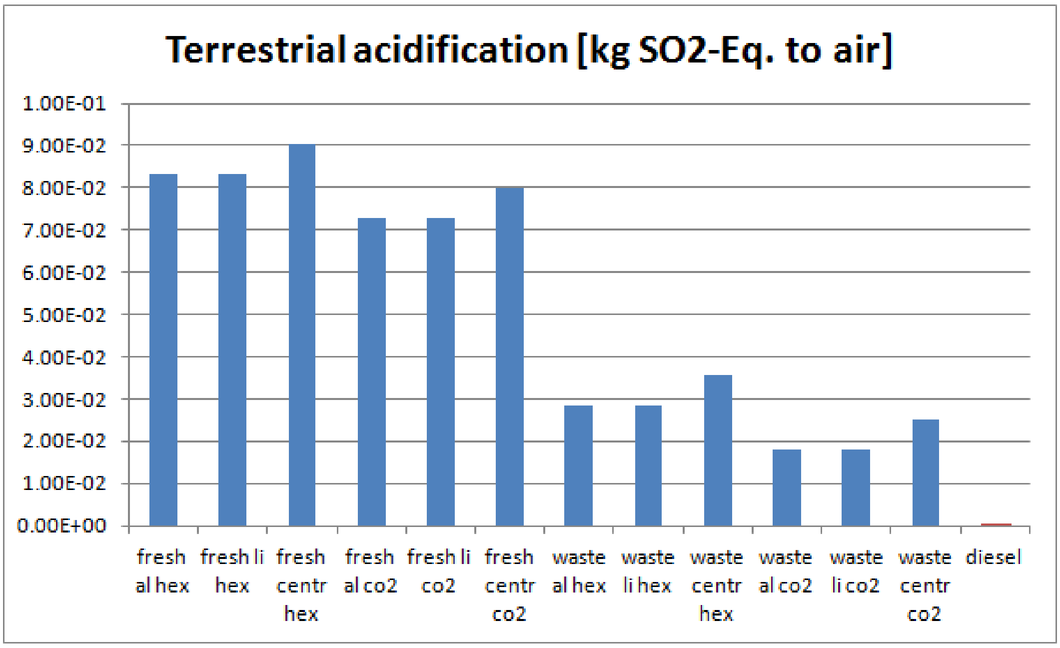


Figure 5.12: terrestrial AP for algal biodiesel is compared to diesel. These scenario are performed with the use of waste CO_2

5.1.7 Carcinogens and non carcinogens

5.1.7.1 Carcinogens

Scenarios for carcinogens are illustrated in Figure 5.13 with the use of CO_2 synthetic while Figure 5.14 shows carcinogens when waste CO_2 is considered. All scenarios are worse than diesel. Both "freshwater and wastewater scenarios" does not present relevant differences (Figure 5.13 and Figure 5.14).

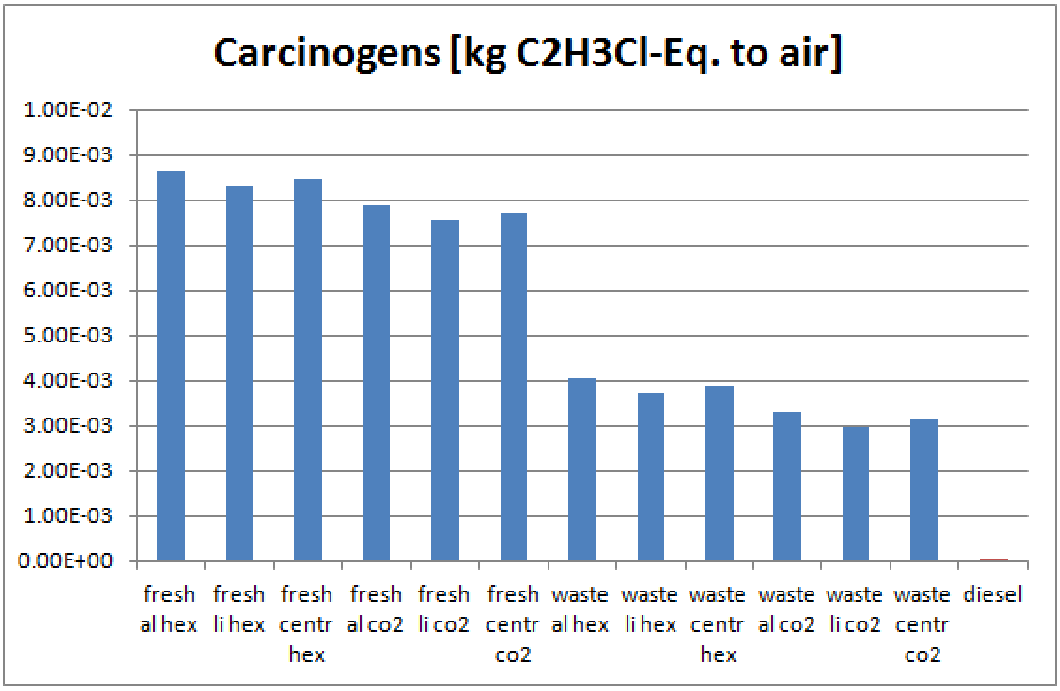


Figure 5.13: carcinogens for algal biodiesel compared to diesel. These scenarios are performed, when synthetic CO_2 is used

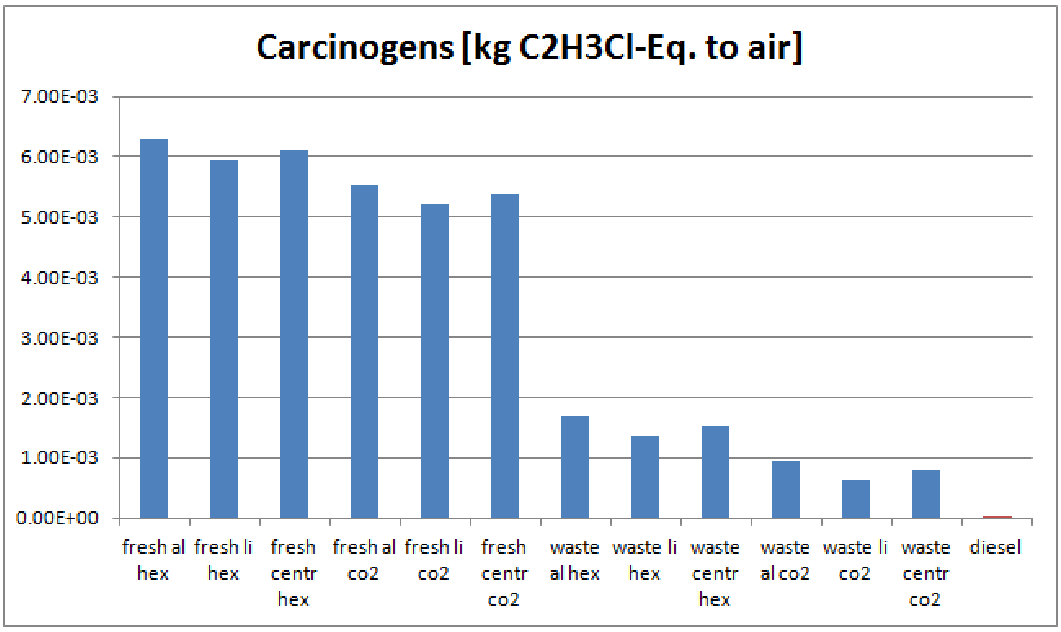


Figure 5.14: carcinogens for algal biodiesel are compared to diesel. These scenarios are performed, when waste CO_2 is used

5.1.7.2 Non carcinogens

Figure 5.15 shows non carcinogens when synthetic CO_2 is used, highlighting that algal biodiesel production has higher impacts than the one of the diesel production.

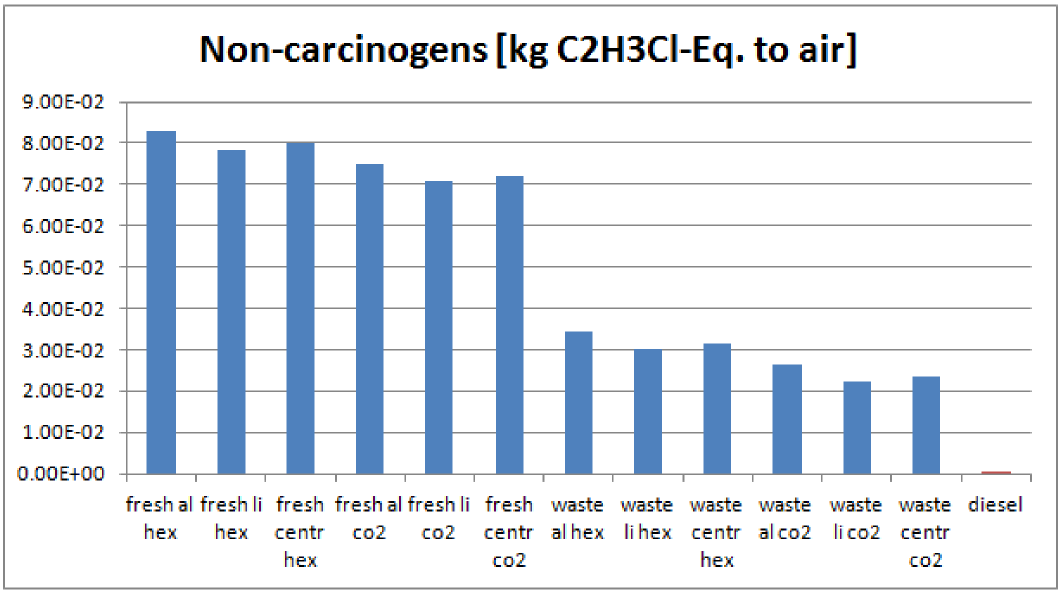


Figure 5.15: non carcinogens for algal biodiesel are compared to diesel. These scenarios are performed, when synthetic CO_2 is used

The use of waste CO_2 for non carcinogens is illustrated in Figure 5.16. For algal biodiesel, non carcinogens are worse than diesel as well.

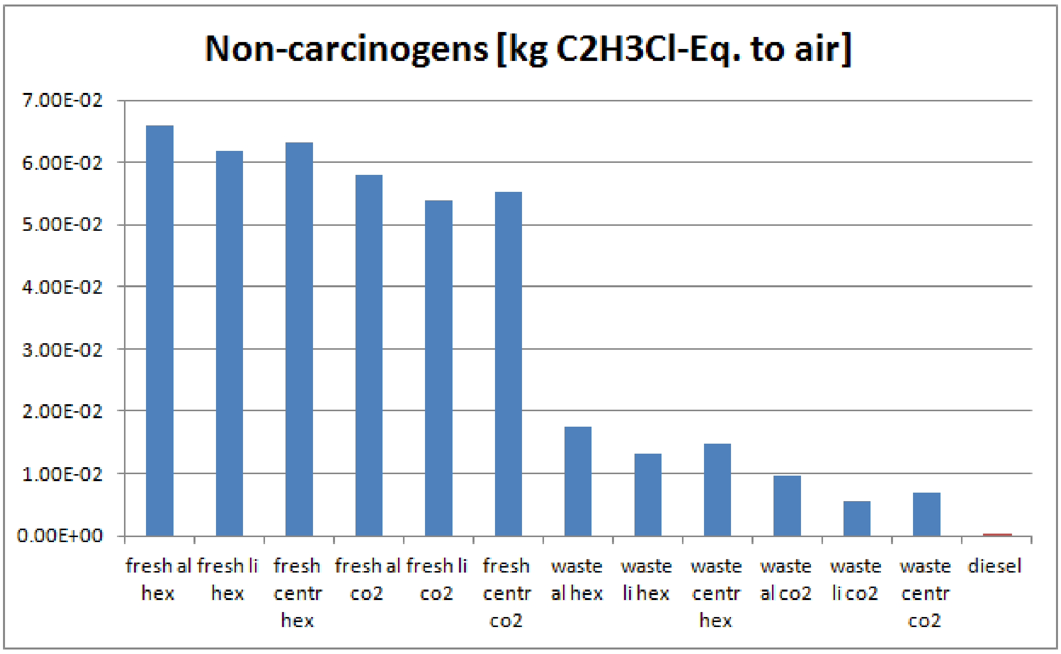


Figure 5.16: non carcinogens for algal biodiesel compared to diesel. These scenarios are performed, when waste CO_2 is used

Obviously, the use of freshwater for algal cultivation impacts more than the use of wastewater. Hence, "wastewater scenarios" show avoided impacts for aquatic eutrophication because wastewater cultivation does not need to the addition of nutrients such as nitrate and phosphate.

The use of waste CO_2 avoids GHG emissions only for the Scenario 4 (sCO_2 extraction and aluminum flocculation) in the case that wastewater cultivation is assessed. In the other scenarios, all impacts are worse than diesel. The use of sCO_2 is used for lipid extraction from wet biomass in fact drying phase is avoided when this technology is applied. It is also important to highlight that GHG emissions saving is reached when waste CO_2 and wastewater are considered but these two options have not been implemented on industrial scale for biodiesel production yet.

In most of the 24 scenarios analyzed, algal biodiesel is worse than diesel, showing a difference of one order of magnitude in non renewable energy consumption, POCP with waste CO_2 and ODP. In HT, POCP with synthetic CO_2, AP and GWP, this difference corresponds to two orders of magnitude while EP differs to three orders of magnitude, compared to diesel.

This analysis provides general considerations about environmental impacts related to this process and how it could be developed on industrial scale. This analysis highlights that technologies have not been developed enough to make algal biodiesel production sustainable on commercial scale. Probably, implementing the use of wastewater and waste CO_2, algal biodiesel production can be improved in terms of environmental impacts and its commercialization could start.

5.2 Scenario “aluminum flocculation and hexane extraction” (scenario 1)

The aim of this section is an analysis of both total and relative contributions of different phases and unit processes to the different impact categories. Different scenarios are described in order to highlight which phases are the most impacting and which unit processes are the most relevant.

As described in section 3.2.5, scenario 1 is carried out with aluminum flocculation coupled with drying phase and hexane extraction. In addition, “freshwater and wastewater scenarios” are alternatively coupled with the use of synthetic CO_2 or waste CO_2 for cultivation of microalgae.

Firstly, the total contributions are generally described by a table which illustrates processes and impact categories for each case.

When the use of synthetic CO_2 and freshwater are considered, the total contributions of each process are shown in Table 5.4. As it is possible to observe, the cultivation phase shows the highest contributions, followed by drying and hexane extraction and aluminum flocculation. Only anaerobic digestion shows negative contributions indicating avoided impacts to all categories. The contributions of transesterification and the use of glycerol are negligible.

Impact categories with synthetic CO_2	CULTIVATION IN FRESHWATER	FLOCCULATION WITH ALUMINUM SULPHATE	DRY AND HEXANE EXTRACTION	ANAEROBIC DIGESTION	TRANSESTERIFICATION	USE OF GLYCEROL
Aquatic acidification	1.43E-02	1.06E-03	2.67E-03	-2.22E-04	6.30E-07	-1.87E-07
Aquatic eutrophication	4.14E-04	1.37E-05	6.95E-06	-5.58E-07	2.64E-08	-2.90E-09
Carcinogens	7.42E-03	4.65E-04	7.75E-04	-3.60E-05	2.37E-07	-2.70E-08
Global warming 500yr	4.49E+00	1.43E-01	1.45E+00	-1.46E-01	5.55E-04	-6.05E-05
Non-carcinogens	6.96E-02	5.30E-03	8.19E-03	-3.05E-04	2.19E-06	-1.15E-06
Non-renewable energy consumption	6.09E+01	2.16E+00	2.15E+01	-1.96E+00	2.41E-02	-1.67E-03
Ozone layer depletion	3.15E-07	9.10E-09	1.69E-07	-7.23E-09	1.25E-10	-3.62E-11
Photochemical oxidation	8.33E-04	2.17E-05	2.60E-04	-1.58E-07	2.31E-07	-4.95E-08
Terrestrial acidification	8.07E-02	2.57E-03	1.16E-02	-7.08E-04	4.73E-06	-8.10E-07

Table 5.4: the total contribution of each process to all categories when synthetic CO_2 and freshwater are considered

Figure 5.17 illustrates the relative contributions of each process to the different impact categories. As it possible to observe, the most impacting process is the cultivation in “freshwater scenario”. In fact its contribution is in a range from 65% to 95%. The impact categories that have low contributions are GWP, ODP and non renewable energy consumption. Cultivation phase impacts the most in EP (for nitrate and phosphate addition to water), carcinogens and POCP. Aluminum flocculation varies from 2% to 5% (aquatic AP, carcinogens and non carcinogens, respectively). This is due to the toxicity of aluminum sulphate. Drying phase and hexane extraction contribute mainly to GWP, ODP, POCP, AP and non renewable energy consumption. Transesterification and glycerol use for glycol propylene have negligible contributions. The negative contribution of anaerobic digestion (AD) means that impacts related to this process are avoided. The avoided impacts are related to the system expansion for biogas production. In fact, the biogas is a co-product used for electricity production. This avoids the same amount of electricity from Danish electricity mix, as stated in section 3.2.3.

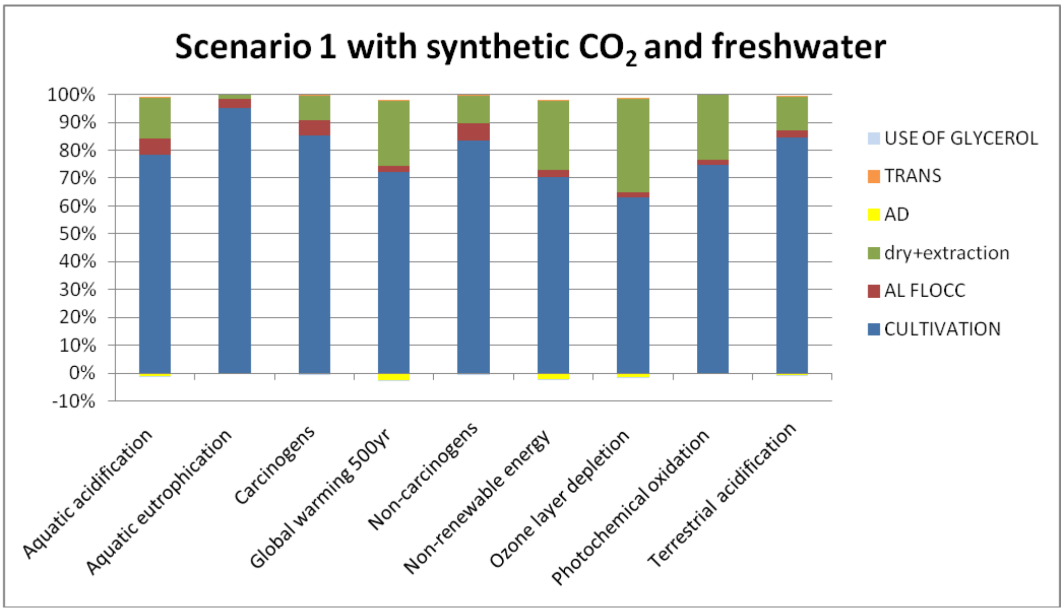


Figure 5.17: contribution of each process to each impact category assuming the use of synthetic CO_2 and freshwater for cultivation. Al flocc describes flocculation with aluminum sulphate, dry+ extraction indicate drying phase and hexane extraction, trans is for transesterification and use of glycerol for the production of glycol propylene. AD indicates the anaerobic digestion. Non renewable energy indicates non renewable energy consumption

Table 5.5 illustrates the total contributions of each process to all categories assuming the use of waste CO_2 and freshwater for cultivation phase. Also in this case, cultivation and drying phase coupled with hexane extraction are the most impacting processes. The other phases analyzed have the same contributions of those illustrated by the Table 5.4.

Impact categories with waste CO_2	CULTIVATION IN FRESHWATER	FLOCCULATION WITH ALUMINUM SULPHATE	DRY AND HEXANE EXTRACTION	ANAEROBIC DIGESTION	TRANSESTERIFICATION	USE OF GLYCEROL
Aquatic acidification	1.18E-02	1.06E-03	2.67E-03	-2.22E-04	6.30E-07	-1.87E-07
Aquatic eutrophication	3.81E-04	1.37E-05	6.95E-06	-5.58E-07	2.64E-08	-2.90E-09
Carcinogens	5.08E-03	4.65E-04	7.75E-04	-3.60E-05	2.37E-07	-2.70E-08
Global warming 500yr	1.66E+00	1.43E-01	1.45E+00	-1.46E-01	5.55E-04	-6.05E-05
Non-carcinogens	5.27E-02	5.30E-03	8.19E-03	-3.05E-04	2.19E-06	-1.15E-06
Non-renewable energy consumption	4.33E+01	2.16E+00	2.15E+01	-1.96E+00	2.41E-02	-1.67E-03
Ozone layer depletion	2.21E-07	9.10E-09	1.69E-07	-7.23E-09	1.25E-10	-3.62E-11
Photochemical oxidation	5.32E-04	2.17E-05	2.60E-04	-1.58E-07	2.31E-07	-4.95E-08
Terrestrial acidification	7.00E-02	2.57E-03	1.16E-02	-7.08E-04	4.73E-06	-8.10E-07

Table 5.5: total contributions of each process when freshwater and waste CO_2 are considered

Figure 5.18 shows the relative contributions of each phase to all environmental impacts. As it is possible to observe, cultivation is the most impacting phase. Due to the addition of nitrate and phosphate in water, this process impacts the most to EP while contributions of cultivation to the other categories corresponds to a range from 50% (GWP) to 80% (HT and terrestrial AP). Excluding EP, also drying phase and hexane extraction have high contributions to all categories. Aluminum flocculation contributes from 1% to 5% to carcinogens and non carcinogens, respectively, as stated in the description of Figure 5.17. Transesterification and glycerol use contributions are negligible. Also in this case, anaerobic digestion avoids impacts in AP, ODP, GWP and non renewable energy consumption. The avoided impacts are related to the system expansion for biogas production.

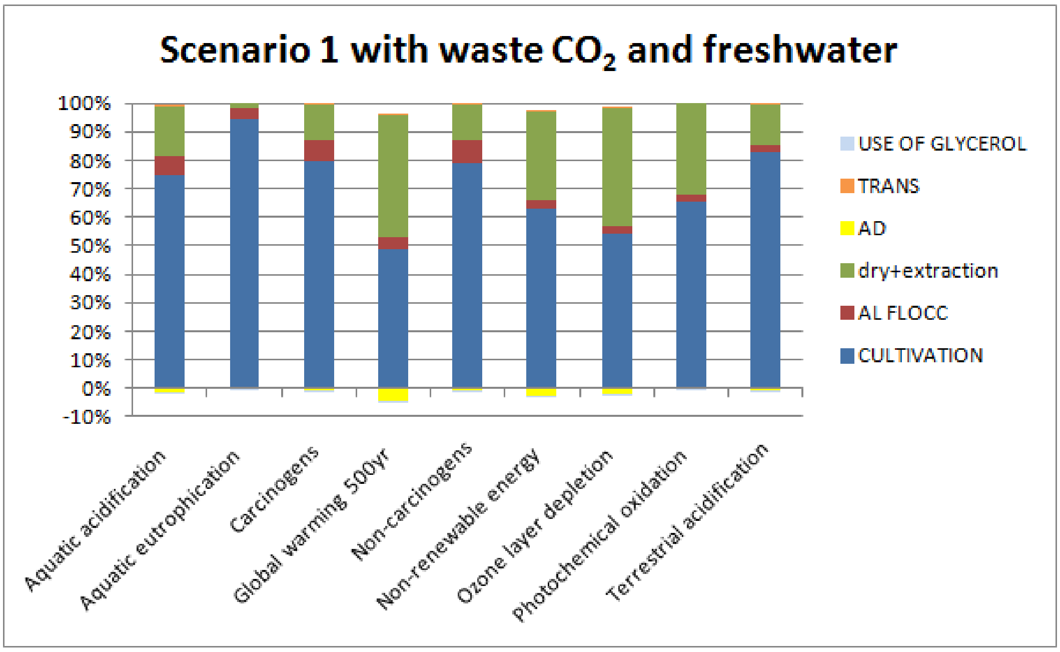


Figure 5.18: contribution of each process to each impact category when waste CO_2 and freshwater for cultivation are used. Al flocc describes flocculation with aluminum sulphate, dry+ extraction indicate drying phase and hexane extraction, trans is for transesterification and use of glycerol for the production of glycol propylene. AD indicates the anaerobic digestion. Non renewable energy indicates non renewable energy consumption

Table 5.6 shows the total contribution of each process to all categories in case that freshwater is replaced by wastewater and the synthetic CO_2 is used. Excluding EP, cultivation phase impacts the most to all categories but the use of wastewater decreases its total contributions. The other phases analyzed have the same contributions of those illustrated by the Table 5.4 and the Table 5.5.

Impact categories with synthetic CO_2	CULTIVATION IN WASTEWATER	FLOCCULATION WITH ALUMINUM SULPHATE	DRY AND HEXANE EXTRACTION	ANAEROBIC DIGESTION	TRANSESTERIFICATION	USE OF GLYCEROL
Aquatic acidification	6.53E-03	1.06E-03	2.67E-03	-2.22E-04	6.30E-07	-1.87E-07
Aquatic eutrophication	-2.22E-01	1.37E-05	6.95E-06	-5.58E-07	2.64E-08	-2.90E-09
Carcinogens	2.83E-03	4.65E-04	7.75E-04	-3.60E-05	2.37E-07	-2.70E-08
Global warming 500yr	2.56E+00	1.43E-01	1.45E+00	-1.46E-01	5.55E-04	-6.05E-05
Non-carcinogens	2.11E-02	5.30E-03	8.19E-03	-3.05E-04	2.19E-06	-1.15E-06
Non-renewable energy consumption	3.76E+01	2.16E+00	2.15E+01	-1.96E+00	2.41E-02	-1.67E-03
Ozone layer depletion	1.72E-07	9.10E-09	1.69E-07	-7.23E-09	1.25E-10	-3.62E-11
Photochemical oxidation	4.96E-04	2.17E-05	2.60E-04	-1.58E-07	2.31E-07	-4.95E-08
Terrestrial acidification	2.59E-02	2.57E-03	1.16E-02	-7.08E-04	4.73E-06	-8.10E-07

Table 5.6: the total contribution of each process to all categories when synthetic CO_2 and wastewater are considered

Figure 5.19 shows the relative contribution of each process to each impact categories. The use of wastewater avoids the addition of nitrate and phosphate to cultivation water. This means avoided impact to EP (-100%). Moreover, the cultivation in wastewater decreases contribution to cultivation for other impact categories such as GWP, non renewable energy consumption and ODP, ranging from 50% to 70% in carcinogens. Drying and hexane extraction impact the most to ODP, GWP, POCP and non renewable energy consumption. In particular for ODP, these two processes have higher contributions than cultivation.

Contribution of aluminum flocculation ranges from 2% (GWP, non renewable energy consumption, ODP and POCP) to 15% in non carcinogens. Transesterification has a negligible contribution to each impact category as well as the use of glycerol for its system expansion. On the other hand, anaerobic digestion has negative contribution to GWP, AP, non renewable energy consumption and ODP.

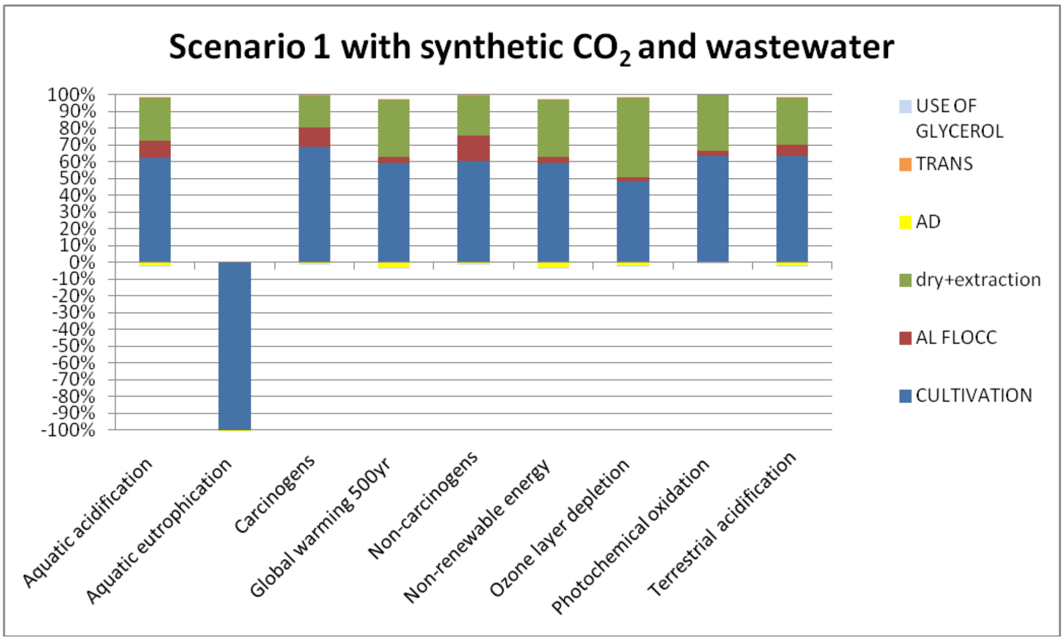
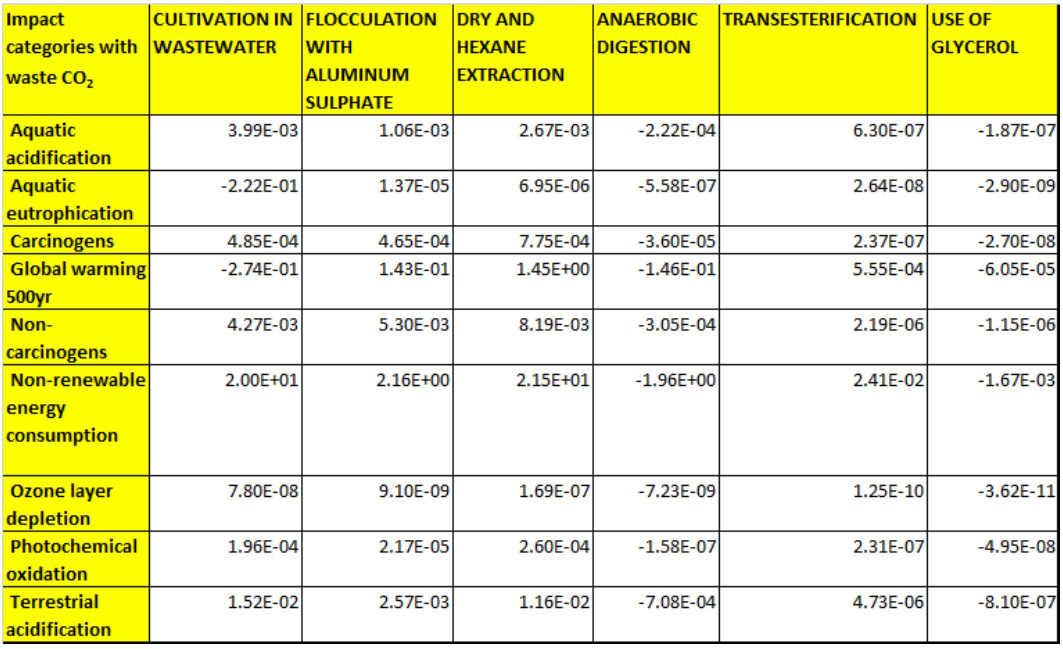


Figure 5.19: contribution of each process to each impact category when synthetic CO_2 is used. Freshwater is replaced by wastewater. Al flocc describes flocculation with aluminum sulphate, dry+ extraction indicate drying phase and hexane extraction, trans is for transesterification and use of glycerol for the production of glycol propylene. AD indicates the anaerobic digestion. Non renewable energy indicates non renewable energy consumption

Table 5.7 analyses the total contributions of each process to all impact categories when wastewater displaces freshwater and synthetic CO_2 is replaced by waste CO_2. The use of waste flows avoids impacts to GWP and EP and it makes drying phase and hexane extraction the most relevant processes. Other phases analyzed have the same contributions of those illustrated by the Table 5.4 and Table 5.5.



Impact categories with waste CO_2	CULTIVATION IN WASTEWATER	FLOCCULATION WITH ALUMINUM SULPHATE	DRY AND HEXANE EXTRACTION	ANAEROBIC DIGESTION	TRANSESTERIFICATION	USE OF GLYCEROL
Aquatic acidification	3.99E-03	1.06E-03	2.67E-03	-2.22E-04	6.30E-07	-1.87E-07
Aquatic eutrophication	-2.22E-01	1.37E-05	6.95E-06	-5.58E-07	2.64E-08	-2.90E-09
Carcinogens	4.85E-04	4.65E-04	7.75E-04	-3.60E-05	2.37E-07	-2.70E-08
Global warming 500yr	-2.74E-01	1.43E-01	1.45E+00	-1.46E-01	5.55E-04	-6.05E-05
Non-carcinogens	4.27E-03	5.30E-03	8.19E-03	-3.05E-04	2.19E-06	-1.15E-06
Non-renewable energy consumption	2.00E+01	2.16E+00	2.15E+01	-1.96E+00	2.41E-02	-1.67E-03
Ozone layer depletion	7.80E-08	9.10E-09	1.69E-07	-7.23E-09	1.25E-10	-3.62E-11
Photochemical oxidation	1.96E-04	2.17E-05	2.60E-04	-1.58E-07	2.31E-07	-4.95E-08
Terrestrial acidification	1.52E-02	2.57E-03	1.16E-02	-7.08E-04	4.73E-06	-8.10E-07

Table 5.7: the total contribution of each process to all categories when waste CO_2 and wastewater are considered

Figure 5.20 shows the relative contribution of each process to each impact categories. The use of wastewater avoids impacts to EP (-100%). Due to the wastewater enrichment in nitrogen and phosphorus, the addition of nutrients is not necessary avoiding impacts related to nitrate, phosphate and tap water production. The use of waste CO_2 increases the relative contributions of drying and hexane extraction to all categories, excluding EP and AP. In particular, these processes have higher impacts than cultivation because the waste CO_2 avoids impacts related to CO_2 production while synthetic CO_2 does not. Hence, contributions of wastewater decrease significantly and the negative contribution of cultivation to GWP (-10%) corresponds to an avoided impact.

In HT, aluminum flocculation has higher contribution than cultivation as well. This is due to the toxicity of aluminum sulphate, used for algal biomass flocculation.

Transesterification has a neglectable contribution for each impact category as well as the use of glycol propylene. Anaerobic digestion has negative impacts except to EP and POCP.

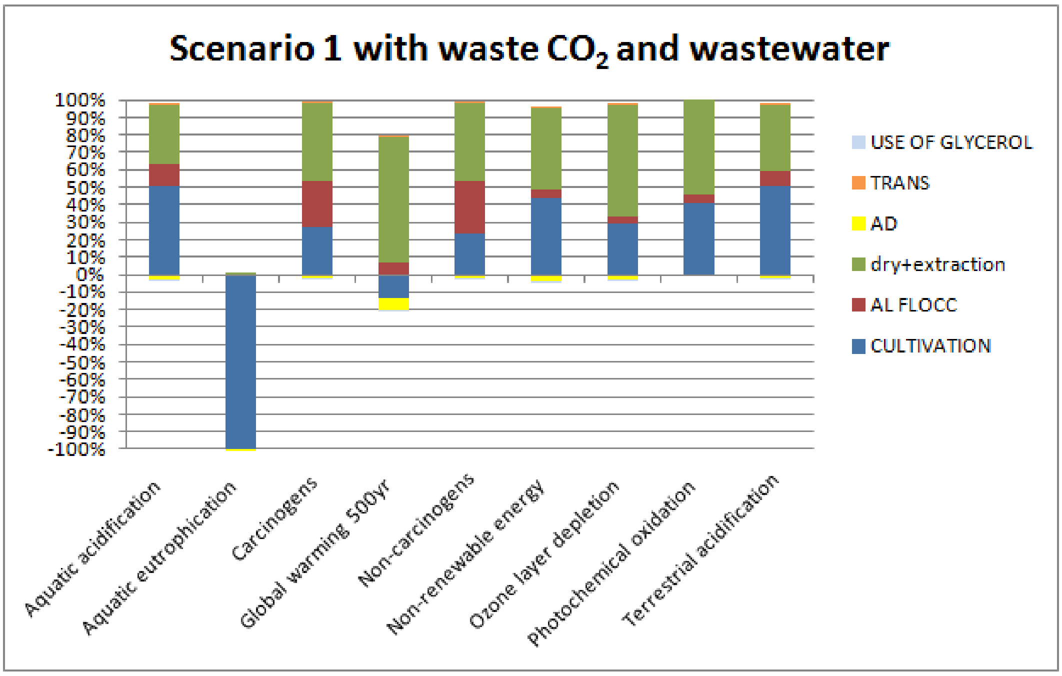


Figure 5.20: contribution of each process to each impact category when waste CO_2 is used. Wastewater displaces the use of freshwater. Al flocc describes flocculation with aluminum sulphate, dry+ extraction indicate drying phase and hexane extraction, trans is for transesterification and use of glycerol for the production of glycol propylene. AD indicates the anaerobic digestion. Non renewable energy indicates non renewable energy consumption

5.2.1 Contribution of each process unit in freshwater cultivation

Each process unit has a different contribution to each impact category. The aim of this section is to highlight which process unit affects mainly each impact category.

Figure 5.21 illustrates contribution of each process unit in freshwater cultivation coupled with synthetic CO_2. Seven process units (product manufacturing) have been considered: tap water, carbon dioxide, single superphosphate, ammonium nitrate, electricity, LDPE and reinforcing steel. As it is possible to observe from the Figure 5.21, five of them are relevant (tap water, carbon dioxide, single superphosphate, ammonium nitrate, electricity) and two of them are always negligible (LPDE, reinforcing steel). Tap water, ammonium nitrate and single superphosphate as nutrients contribute mainly to different impact categories while synthetic CO_2 and electricity show a similar contribution to all impact categories.

In particular, it is possible to see that, due to the use of single superphosphate as nutrient, PO_4^{3-} has the highest contribution in EP (80%) whereas tap water, synthetic CO_2 (10%), ammonium nitrate and electricity have not relevant contributions to EP. Tap water contributes the most to

HT. This is due to the process for its production. In fact, this process unit also considers impacts for tap water production process. Ammonium nitrate shows high contributions to AP, GWP and ODP. The use of electricity impacts the most aquatic AP, GWP, non renewable energy consumption and ODP. This is due to the electricity mix composition, as shown in Figure 4.1. POCP, ODP, AP, non renewable energy consumption, GWP and carcinogens are affected by the use of synthetic CO_2 and its production process.

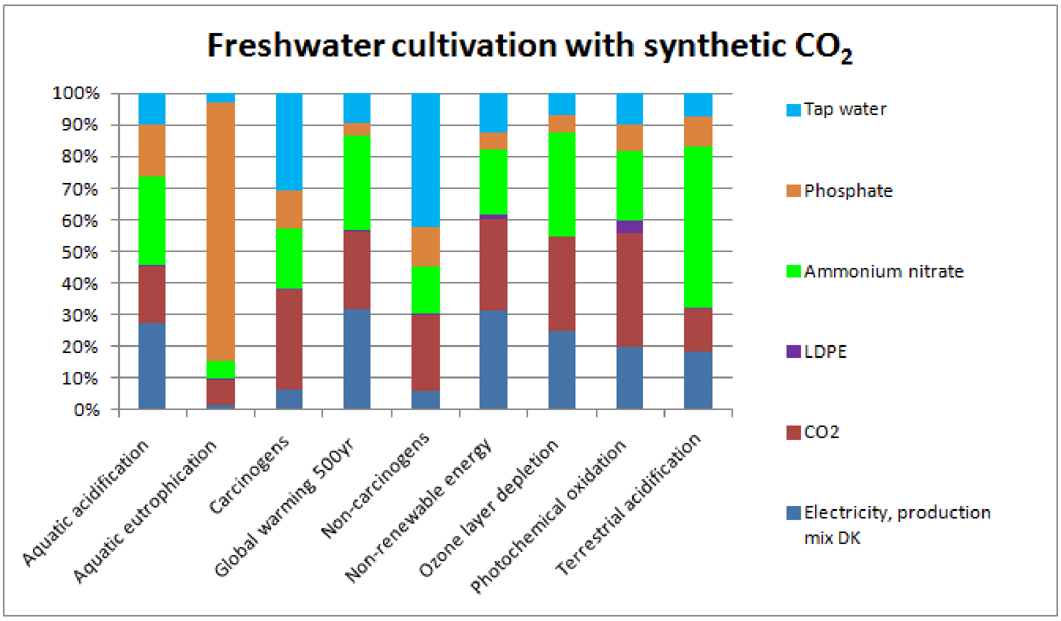


Figure 5.21: contribution of each process unit in freshwater cultivation when synthetic CO_2 is used. Non renewable energy indicates non renewable energy consumption

Figure 5.22 illustrates contribution of each process unit in freshwater cultivation coupled with waste CO_2. In this case four are the process units relevant: tap water, ammonium nitrate, single superphosphate and electricity while LDPE and reinforcing steel have not relevant contributions.

Obviously, the use of waste CO_2 avoids its contribution in GWP (-35%). In fact, the production process of CO_2 is not taken into account because it is a waste flow. This process unit takes in consideration only the flow but neither its production process nor its impacts. In non renewable energy consumption, electricity has a contribution of 45%. Ammonium nitrate provides a contribution of 60% in terrestrial AP. Other process units approximately contribute to all impact categories with the same rate described for Figure 5.21.

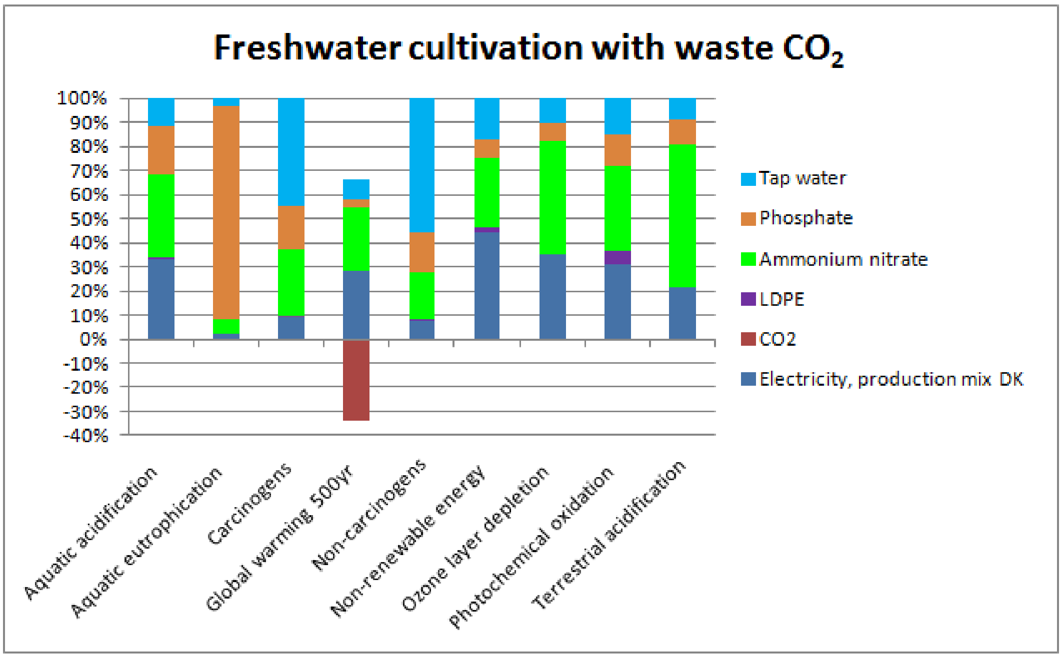


Figure 5.22: contribution of each process unit in freshwater cultivation when waste CO_2 is used. Non renewable energy indicates non renewable energy consumption

5.2.2 Contribution of each process unit in wastewater cultivation

While section 5.2.1 describes the contribution of each process unit when the algae cultivation is practiced in freshwater, in this section the use of wastewater and contribution of each process are analyzed. As investigated in the previous section, the use of synthetic and waste CO_2 are alternatively coupled with wastewater use for cultivation.

In Figure 5.23, the relative contribution of each process unit is shown. The use of wastewater implies that the adding of nitrate and phosphate is avoided as well as the use of tap water in EP (-100%). As it is possible to observe, in this case the electricity consumption and the use of synthetic CO_2 contribute the most to this process. As electricity contributions are the highest for AP, GWP and non renewable energy consumption. On the other hand, synthetic CO_2 impacts the most for HT, ODP and POCP.

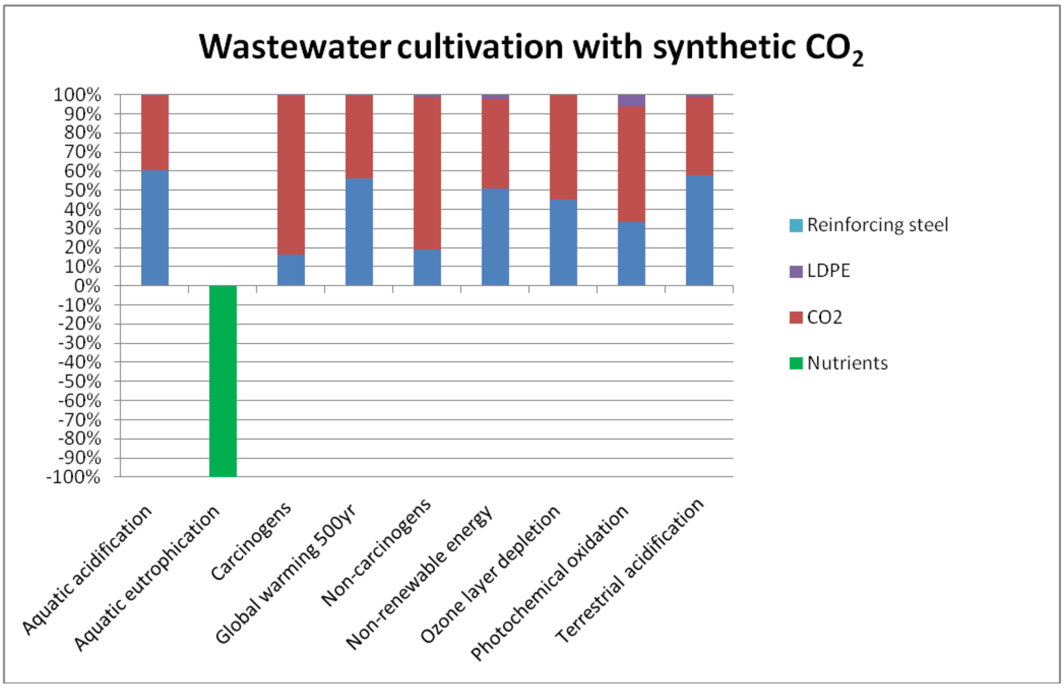


Figure 5.23: contribution of each process unit in wastewater cultivation coupled with the use of synthetic CO_2. Non renewable energy indicates non renewable energy consumption. Nutrients indicate the avoided impact for nitrate, phosphate and tap water

Figure 5.24 illustrates contributions of each process unit when algae cultivation in wastewater is coupled with the use of waste CO_2. This means that specific production of CO_2 for algal growing is avoided as well as the use of nitrate, phosphate and tap water and their production processes. In this case, electricity contributes the most for all impact categories and CO_2 has negative contributions for EP (-100%) and GWP (-55%). In other impact categories, waste CO_2 contribution is neglectable.

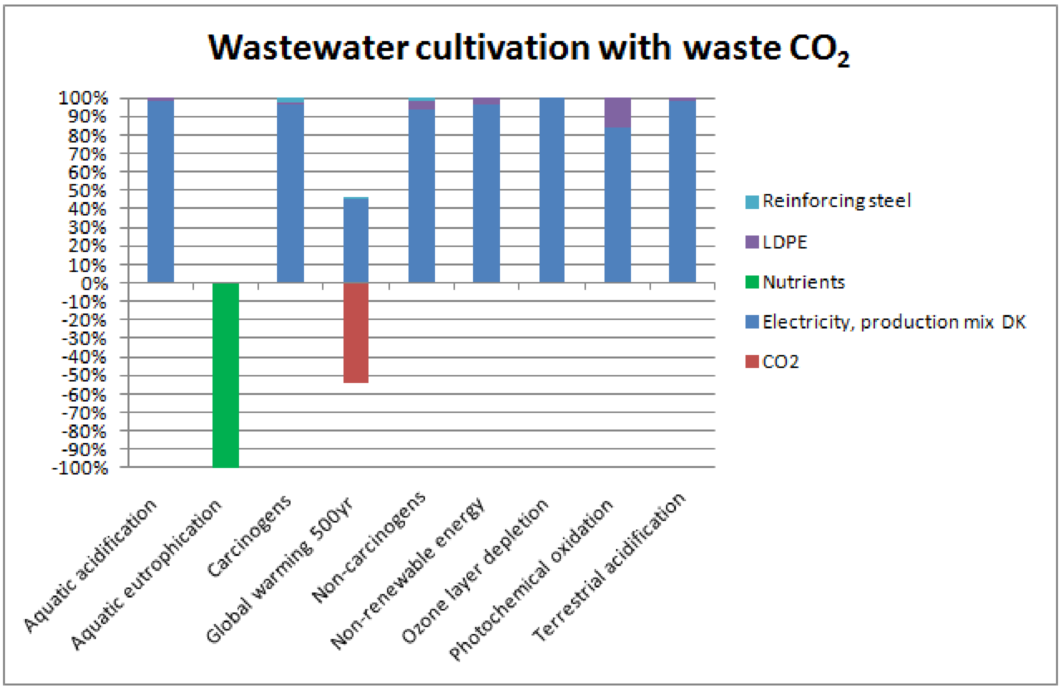


Figure 5.24: contribution of each process unit in wastewater cultivation coupled with the use of waste CO_2. Non renewable energy indicates non renewable energy consumption. Nutrients indicate the avoided impact for nitrate, phosphate and tap water

5.2.3 Contribution of each process unit in aluminum flocculation

The process of flocculation is constituted by two process units: aluminum sulphate production and consumption of electricity.

Figure 5.25 shows the contributions of the two units to each impact category. As it is possible to observe, both have a relevant role. Electricity consumption impacts the most in GWP (65%), non renewable energy consumption (60%), ODP (55%) and POCP (50%). This is attributable to the composition of Danish electricity production mix. On the other hand, aluminum sulphate contributes the most to aquatic AP (75%), EP, carcinogens and non carcinogens (95%) and terrestrial AP (65%).

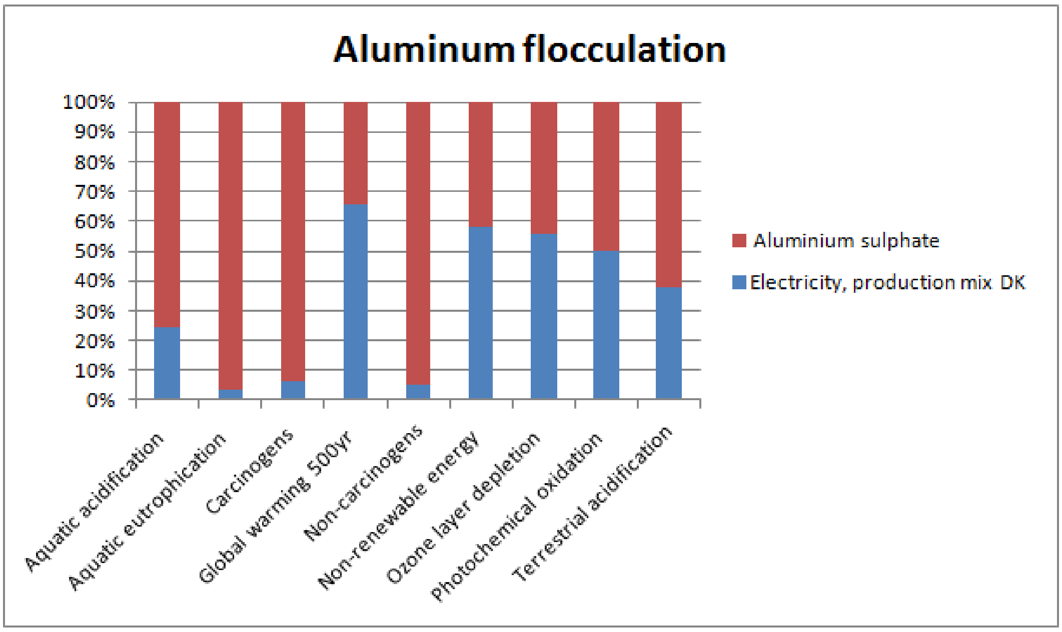


Figure 5.25: contribution of each process unit in aluminum flocculation. Non renewable energy indicates non renewable energy consumption

5.2.4 Contribution of each process unit in hexane extraction

Process units occurring in hexane extraction phase are: thermal energy, electricity consumption and hexane synthesis. The relative contribution to the different impact categories are illustrated in Figure 5.26. The synthesis process of hexane (EP, POCP, ODP) and heat (GWP, AP and HT) show the highest contributions in each impact category. The percent contribution of heat varies from 15% in POCP to 55% in GWP. Hexane impacts the most in POCP (85%). Electricity has important contributions in AP and GWP.

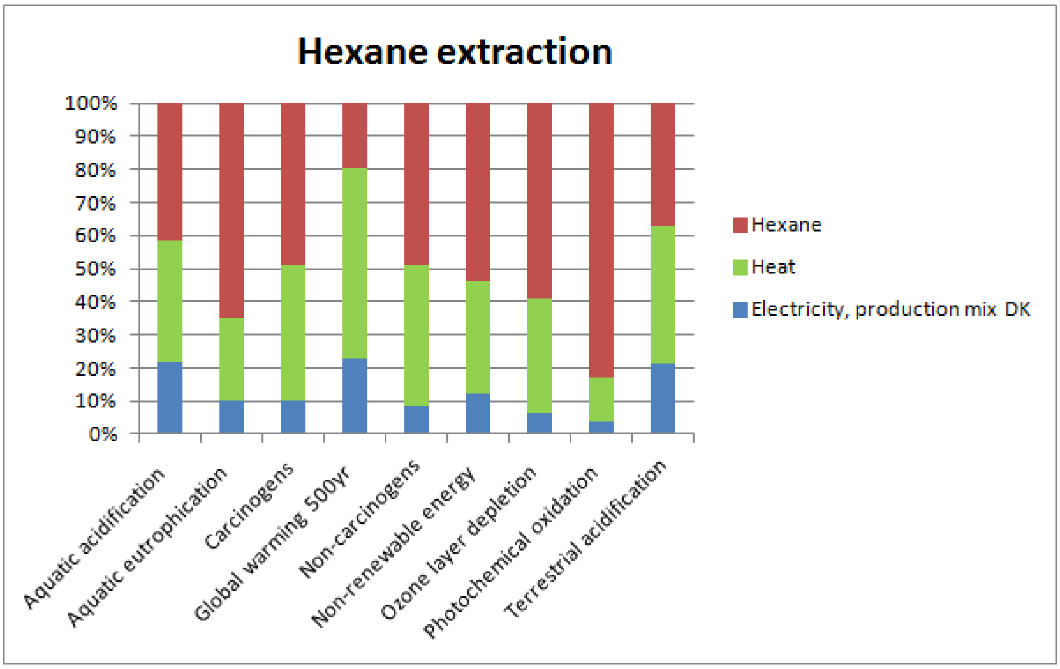


Figure 5.26: contribution of each process unit in hexane extraction. Non renewable energy indicates non renewable energy consumption

5.2.5 Contribution of each process unit in transesterification

Transesterification process is composed by three process unit: thermal energy, methanol as chemical (CH_3OH) and electricity. Figure 5.27 shows the relative contributions of each process unit to each impact category. Heat and CH_3OH has the highest contributions to all impact categories while electricity has not a contribution as relevant as the other two process unit. Particularly, CH_3OH contributes the most to EP (85%), non renewable energy consumption (70%), ODP (60%) and POCP (65%). Contribution of electricity is the highest in aquatic AP (25%). Heat impacts the most aquatic AP, carcinogens, GWP and non carcinogens (45%-55%).

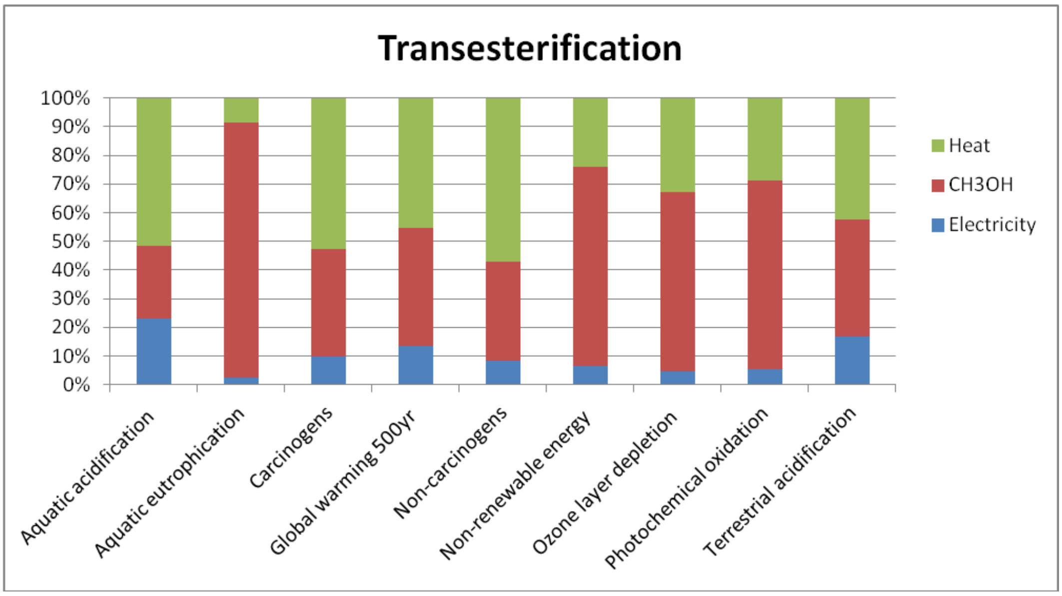


Figure 5.27: contribution of each process unit in transesterification. Non renewable energy indicates non renewable energy consumption

Related to this analysis, some main issues can be discussed as follows:

1. In all four scenarios described, **the cultivation phase plays an important role** on all impact categories. Other relevant processes are drying phase and hexane extraction. **The contribution of aluminum flocculation depends on which impact category is considered**. Its contribution is significant to toxicity and acidification while it is less relevant to other impact category. Transesterification and glycerol use have negligible contributions. **The anaerobic digestion of residual biomass always avoids impacts** in GWP, non renewable energy consumption, ODP and AP.

2. **The relative weight of the cultivation phase changes only in the last scenario**, i.e. when waste CO_2 and wastewater replace freshwater and synthetic CO_2. In this case, the contributions of flocculation, drying phase and hexane extraction are very relevant and higher than cultivation in some categories such as HT, GWP and ODP. In particular, cultivation process avoids impacts for GWP (-10%) and EP (-100%).

3. **Drying phase and hexane extraction require a large thermal energy demand**.

4. In order to make the biodiesel production sustainable, it is necessary **to improve the environmental performance of cultivation and drying phase**. The best case corresponds to the last hypothetic scenario. Improvements in the use of wastewater and waste CO_2 must be achieved. To date, different projects have been implemented in order to work on industrial scale in the next 5-10 years (for example in Cadice and Alicante).

5.3 Scenario "aluminum flocculation and supercritical CO_2 extraction (scenario 4)"

In this section, an analysis of supercritical CO_2 extraction (sCO_2) coupled with "freshwater scenario" and aluminum flocculation (scenario 4) is carried out. For the cultivation, both synthetic and waste CO_2 are considered creating two different scenarios. Figure 5.28 shows the relative contributions of each process analyzed: algae cultivation, aluminum flocculation, sCO_2 extraction, anaerobic digestion, transesterification and glycerol use. As it is possible to observe, algae cultivation is the process unit more relevant. The contributions of the other units result very low. Also in this case, anaerobic digestion results in avoided impacts for GWP, non renewable energy consumption, ODP and AP. Freshwater cultivation impacts by about 90%. Aluminum flocculation varies from 5% (GWP, non renewable energy consumption and ODP) to 10% (aquatic AP, carcinogens and non carcinogens). In contrast to scenario 1 (section 5.2), the use of sCO_2 extraction implies that drying phase is avoided (as stated in section 2). This means a lower contribution of this process to the different impact categories such as GWP, AP and POCP. For anaerobic digestion, contributions for each impact category are equal to those illustrated in Figure 5.17. Transesterification and glycerol use result negligible.

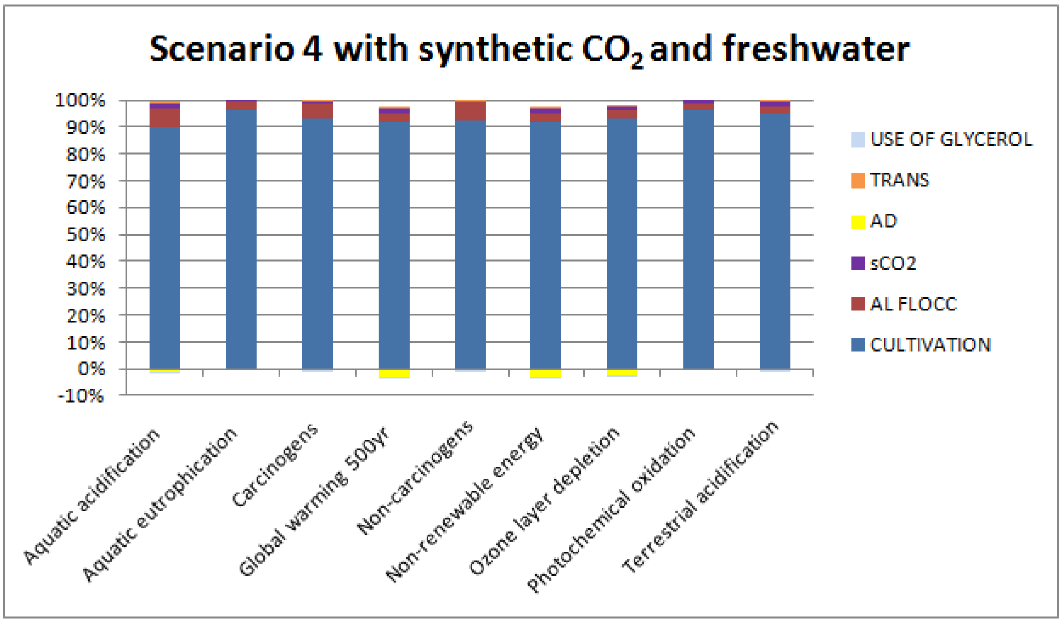


Figure 5.28: contribution of different processes to each impact category. sCO_2 extraction is performed with freshwater and synthetic CO_2 use. Al flocc describes flocculation with aluminum sulphate, sCO_2 indicates sCO_2 extraction, trans is for transesterification and use of glycerol for the production of glycol propylene. AD

indicates the anaerobic digestion. Non renewable energy indicates non renewable energy consumption

Figure 5.29 shows contributions of each process when scenario 4 considers the use of waste CO_2 for the algae cultivation. Cultivation is the most impacting process unit, followed by aluminum flocculation and sCO_2 extraction. In particular, cultivation contributes mainly in a range from 80% (GWP) to 95% (EP).

As it is possible to observe, anaerobic digestion contributes to avoid impact in GWP, ODP and non renewable energy consumption, corresponding to a range from -1% in aquatic AP to -10% in GWP.

Depending on impact category analyzed, the contribution of aluminum flocculation varies a lot. In aquatic AP, GWP, carcinogens and non carcinogens, its contribution is relevant whereas it is less significant in non renewable energy consumption, ODP, POCP, terrestrial AP and EP.

In contrast to drying phase and hexane extraction, sCO_2 extraction contributes from 1% (carcinogens and non carcinogens) to 5% in GWP.

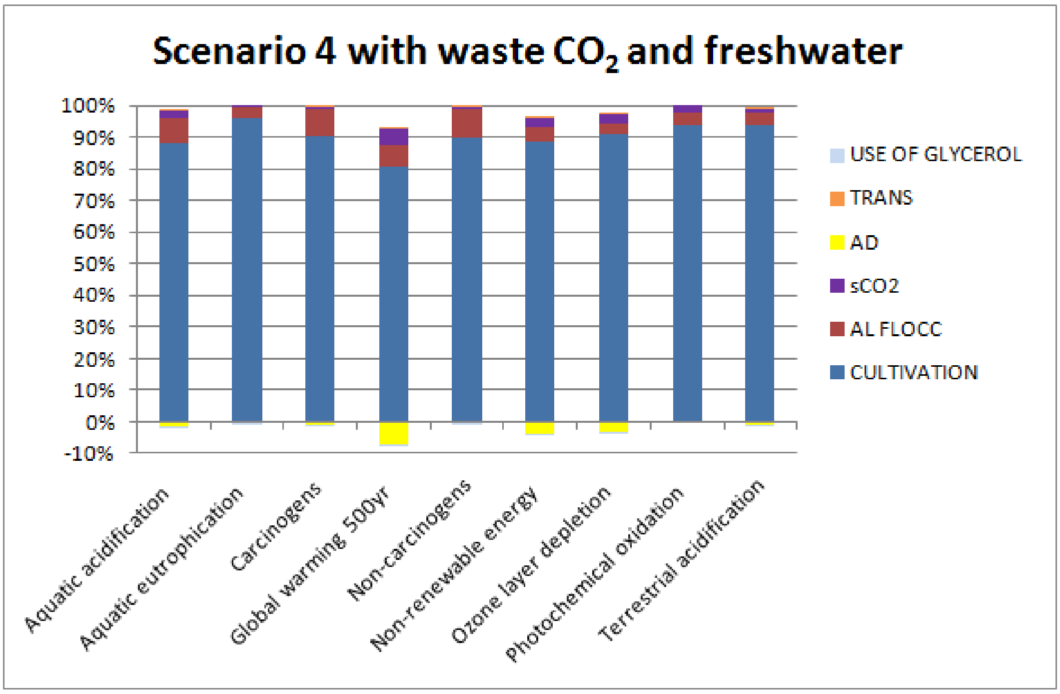


Figure 5.29: contribution of different processes to each impact category. sCO_2 extraction is performed with freshwater and waste CO_2 use. Al flocc describes flocculation with aluminum sulphate, sCO_2 indicates sCO_2 extraction, trans is for transesterification and use of glycerol for the production of glycol propylene. AD indicates the anaerobic digestion. Non renewable energy indicates non renewable energy consumption

As performed for scenario 1 (see section 5.2), wastewater for algae cultivation displaces freshwater. Both synthetic CO_2 and waste CO_2 are considered, modeling two different scenarios.

Figure 5.30 shows the contribution of each process to each impact categories in the case that freshwater is replaced by wastewater and synthetic CO_2 is used for microalgae cultivation. The process units analyzed are algae cultivation, aluminum flocculation, sCO_2 extraction, anaerobic digestion, transesterification and glycerol use. Except to EP, cultivation contributes the most to all impact categories. The use of wastewater avoids the addition of nitrate and phosphate to cultivation water. This means avoided impact to EP (-100%) and a decrease of cultivation contributions to aquatic AP, GWP, ODP, POCP, terrestrial AP, carcinogens, non carcinogens and non renewable energy consumption. Other relevant contributions correspond to aluminum flocculation. Aluminum flocculation contributes the most to carcinogens, non carcinogens and aquatic AP. sCO_2 extraction impacts mainly to aquatic and terrestrial AP, GWP, non renewable energy consumption, ODP and POCP by about 2% on average. Transesterification and glycerol use have a negligible contributions to all impact categories. Also in this case, contributions of anaerobic digestion correspond to avoided impacts in all categories.

Compared to Figure 5.19, aluminum flocculation (from 5% to 20%) and cultivation (75%-90 in POCP) have higher contributions for each process than the ones of the freshwater scenario. On the other hand, due to the avoided drying phase, sCO_2 extraction contributes less than drying and hexane extraction. In fact, its contributions vary from 2% to 5% in POCP, GWP and aquatic and terrestrial AP. In anaerobic digestion, there is a saving of GHG emissions corresponding to negative values in GWP, HT, ODP, AP and non renewable energy consumption.

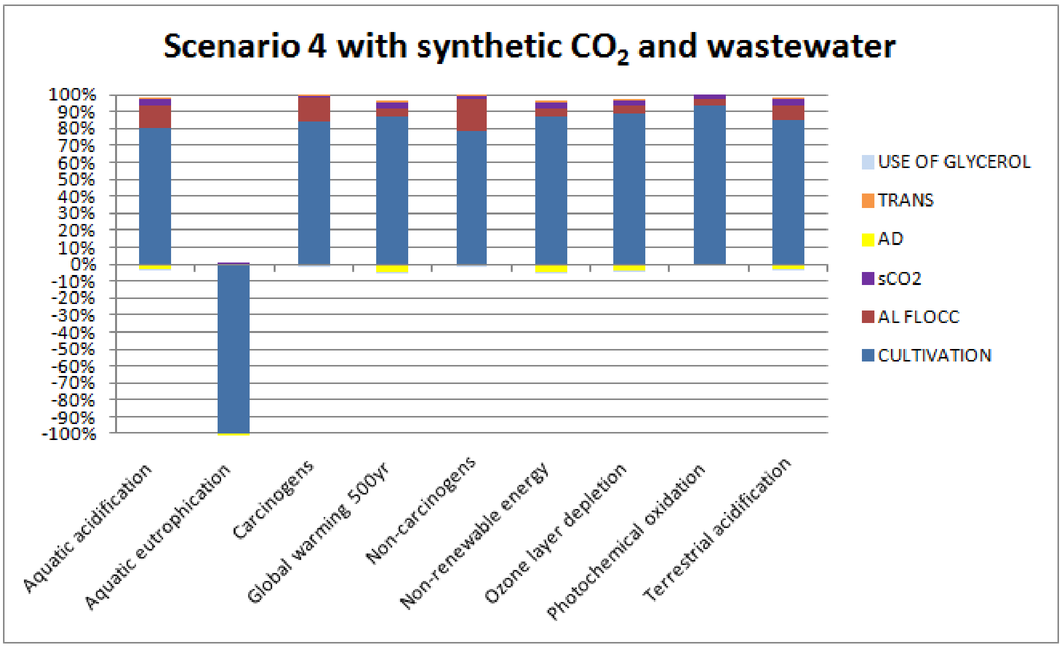


Figure 5.30: contribution of different processes to each impact category. sCO_2 extraction is performed with wastewater and synthetic CO_2 use. Al flocc describes flocculation with aluminum sulphate, sCO_2 indicates sCO_2 extraction, trans is for transesterification and use of glycerol for the production of glycol propylene. AD indicates the anaerobic digestion. Non renewable energy indicates non renewable energy consumption

Figure 5.31 shows the relative contributions of each unit process in case that waste CO_2 and the use of wastewater are considered. The use of waste CO_2 decreases contributions of algae cultivation to all impact categories and avoids GHG emissions (-10%). In consequence to this decrease, contributions of aluminum flocculation are the highest ones, followed by sCO_2 extraction. Also in this case, wastewater use contributes to avoid impact in EP (-100%). Contributions of anaerobic digestion are negative, corresponding to avoided impacts in all categories excluding POCP and EP. Cultivation shows a range from -40% in GWP to 85% in POCP. Aluminum flocculation has contributions, varying from 10% in ODP and non renewable energy consumption to 55% in non carcinogens. In particular, flocculation has the highest contributions to HT and GWP. Anaerobic digestion contributes from -2% (carcinogens, aquatic and terrestrial AP) to -20% in GWP. The sCO_2 extraction ranges from 2% to 15% in GWP. This is lower than drying and hexane extraction because algal oil is directly extracted by wet biomass. This means that drying is avoided.

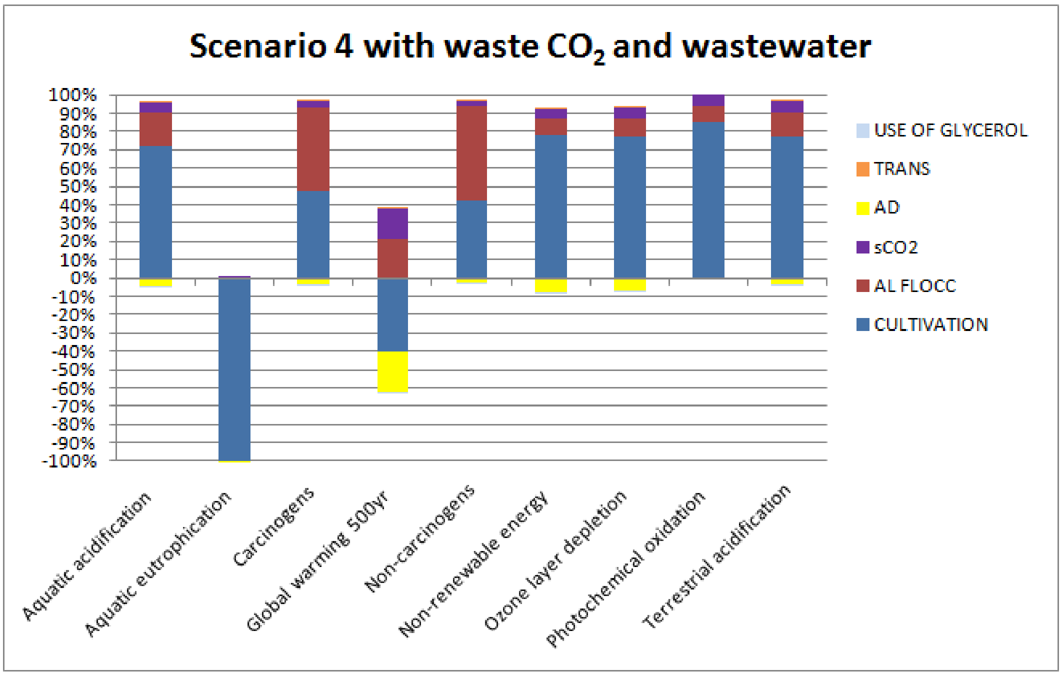


Figure 5.31: contribution of different processes to each impact category. sCO_2 extraction is performed with wastewater and waste CO_2 use. Al flocc describes flocculation with aluminum sulphate, sCO_2 indicates sCO_2 extraction, trans is for transesterification and use of glycerol for the production of glycol propylene. AD indicates the anaerobic digestion. Non renewable energy indicates non renewable energy consumption

Some important issues can be stated after this analysis:

1. In all four scenarios, **cultivation is the most relevant phase** in terms of environmental performance. The contribution of aluminum flocculation varies depending on the impact category considered. Its contribution is significant to human toxicity and AP in all scenarios. In contrast to drying and hexane extraction, **sCO_2 extraction contributes less** to all impact categories. Avoiding drying phase, its relative weight cannot be relevant in some categories, i.e. human toxicity. Obviously, **anaerobic digestion avoids impacts in all categories**.

2. **The relative contribution of cultivation is low assuming the use of wastewater and waste CO_2**. In this case, its relative weight reaches a negative value in GWP. As a consequence, aluminum flocculation and sCO_2 extraction increase their relative weights. In HT and, aluminum flocculation contributions are higher than that of cultivation. Also in GWP, aluminum flocculation and sCO_2 extraction have higher contributions than that of the cultivation phase.

3. **sCO_2 extraction must be developed for this production system**. The main limitation of sCO_2 extraction is related to high pressure. As stated

by Santana and coauthors (2012), an increase of pressure increases the amount of unsaturated compounds and degree of unsaturation in the algal oil. Their presence in the algal oil can decrease the biodiesel quality after transesterification (see section 1.2.1).

4. In order to develop biodiesel production, **cultivation phase and sCO_2 extraction need to be improved** in terms of environmental performance. Although sCO_2 extraction must be developed, the wet extraction shows some favorable aspects such as an avoided drying phase. Therefore, in order to make the biodiesel production sustainable, **the wet extraction coupled with the use of waste CO_2 and the wastewater could be an interesting option**.

6 INTERPRETATION OF RESULTS

In this section, LCIA results are analyzed and discussed. The aim of this chapter is based on a comparison among all scenarios.

A normalization of LCIA results was carried out. “Freshwater, aluminum flocculation and hexane extraction” (basic scenario) is set to 100% in Table 6.1 and in Figure 6.1. Others scenarios are normalized respect to it. In the basic scenario synthetic CO_2 is used. In Figure 6.1, EP is not included; this is because, for this impact category, the others scenarios differ to the basic scenario for three order of magnitude, as shown in Table 6.1. Therefore, should be impossible to show the EP values in the figure. Due to the use of wastewater, EP shows negative normalized values, corresponding to avoided impact because ammonium nitrate and single superphosphate are not added in wastewater. In freshwater scenarios, EP has lower impact than basic scenario.

As it is possible to observe from Figure 6.1, the difference among “freshwater and wastewater scenarios” varies depending on impact category but they have the same trend. In AP and in HT, this difference is by about 60% while in GWP, non renewable energy consumption, ODP and POCP it corresponds to 40% on average.

Since “freshwater and wastewater scenarios” have the same trend, only the group of scenarios assuming algae cultivation in freshwater will be described in the following analysis. Obviously, these considerations are qualitatively the same as those that should be done for the group of scenarios that assume the use of wastewater for cultivation.

Table 6.1 shows impacts higher than basic scenario in red and impacts lower than basic scenario in green.

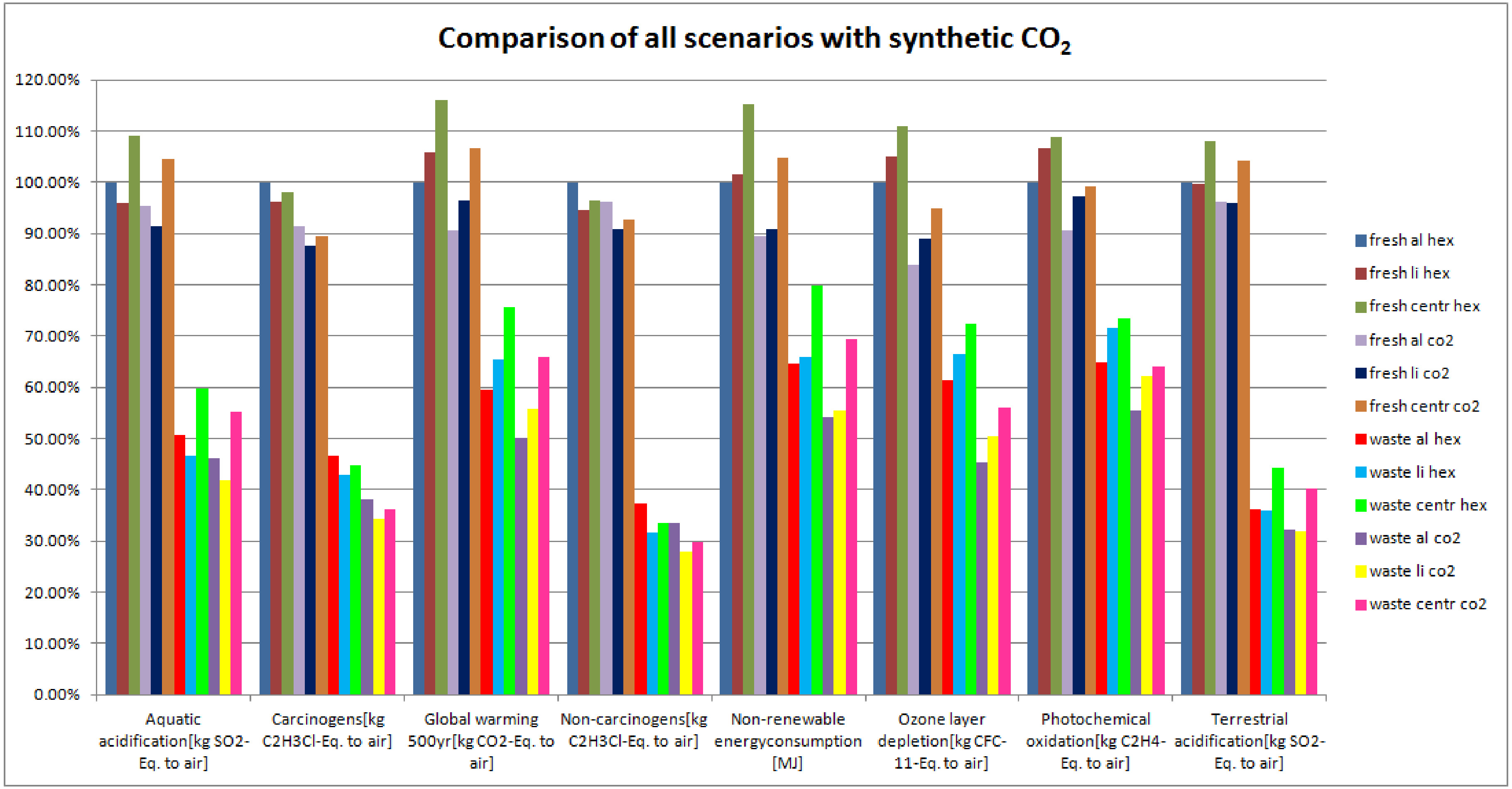
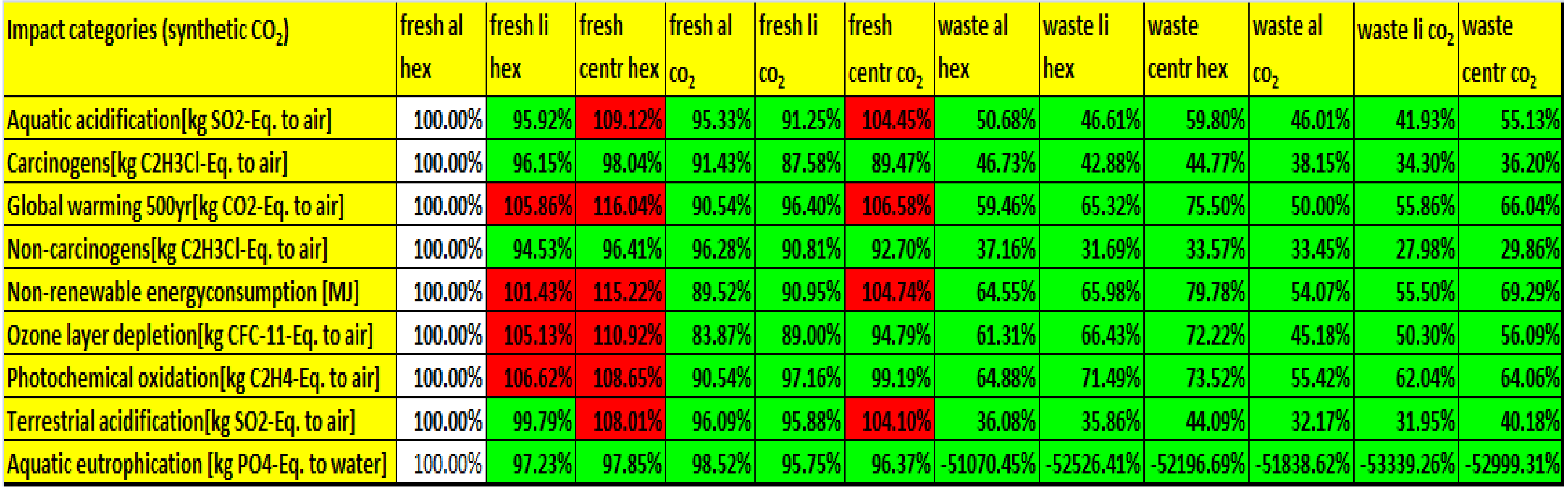


Figure 6.1: "freshwater, aluminum flocculation and hexane extraction" is the basic scenario (100%). Others scenarios are compared to it. EP is not included because the difference between basic scenario and scenarios with the use of wastewater is more than one order of magnitude.



Impact categories (synthetic CO_2)	fresh al hex	fresh li hex	fresh centr hex	fresh al co_2	fresh li co_2	fresh centr co_2	waste al hex	waste li hex	waste centr hex	waste al co_2	waste li co_2	waste centr co_2
Aquatic acidification[kg SO2-Eq. to air]	100.00%	95.92%	109.12%	95.33%	91.25%	104.45%	50.68%	46.61%	59.80%	46.01%	41.93%	55.13%
Carcinogens[kg C2H3Cl-Eq. to air]	100.00%	96.15%	98.04%	91.43%	87.58%	89.47%	46.73%	42.88%	44.77%	38.15%	34.30%	36.20%
Global warming 500yr[kg CO2-Eq. to air]	100.00%	105.86%	116.04%	90.54%	96.40%	106.58%	59.46%	65.32%	75.50%	50.00%	55.86%	66.04%
Non-carcinogens[kg C2H3Cl-Eq. to air]	100.00%	94.53%	96.41%	96.28%	90.81%	92.70%	37.16%	31.69%	33.57%	33.45%	27.98%	29.86%
Non-renewable energyconsumption [MJ]	100.00%	101.43%	115.22%	89.52%	90.95%	104.74%	64.55%	65.98%	79.78%	54.07%	55.50%	69.29%
Ozone layer depletion[kg CFC-11-Eq. to air]	100.00%	105.13%	110.92%	83.87%	89.00%	94.79%	61.31%	66.43%	72.22%	45.18%	50.30%	56.09%
Photochemical oxidation[kg C2H4-Eq. to air]	100.00%	106.62%	108.65%	90.54%	97.16%	99.19%	64.88%	71.49%	73.52%	55.42%	62.04%	64.06%
Terrestrial acidification[kg SO2-Eq. to air]	100.00%	99.79%	108.01%	96.09%	95.88%	104.10%	36.08%	35.86%	44.09%	32.17%	31.95%	40.18%
Aquatic eutrophication [kg PO4-Eq. to water]	100.00%	97.23%	97.85%	98.52%	95.75%	96.37%	-51070.45%	-52526.41%	-52196.69%	-51838.62%	-53339.26%	-52999.31%

Table 6.1: normalization of LCIA results. These results are performed with the use of synthetic CO_2. “Freshwater, aluminum flocculation and hexane extraction” is basic scenario. For this reason, basic scenario is set to 100%. Others scenarios are normalized respect to “freshwater, aluminum flocculation and hexane extraction”. Scenarios worse than the basic are in red whereas scenarios better than basic are in green. In EP, the scenarios with the use of wastewater shows a difference of three orders of magnitude compared with the basic one

As it is possible to observe, the use of centrifugation is the worst option for all scenarios and for all impact categories, excluding carcinogens and non carcinogens. This is due to the high requirement of electricity that characterizes the centrifugation.

Despite its high energy demand, centrifugation is one of the most used methods for algae harvesting because it is currently commercially used and it represents a mature technology (Batan et al., 2010). To date, all full-scale algae production facilities are designed for the extraction of nutriceuticals, or nutrient supplements. In these facilities, centrifugation is convenient even if energy intensive and costly (Brentner et al., 2011) because of the economic value of the end product is very high.

The energy demands reported in the literature for the centrifugation vary from 0.15 MJ/kg of algal biomass (Collet et al. (2011)) to 15 MJ/kg of algal biomass (Sturm and Lamer (2011)). In Table 6.2, literature data are shown. For this work, the energy demand is 5.2 MJ/ kg of algal biomass (Brentner et al., 2011) and it is in the same range of the amounts in others cases studies analyzed in section 1.7. However, *Nannochloropsis* is not a suitable strain for this harvesting technology due to its small cells size.

Authors	**Energy demand for centrifugation**
Batan et al., 2010	10.7 MJ/ kg of algal biomass
Sander and Murthy, 2010	6.2 MJ/ kg of algal biomass
Collet et al., 2011	0.15 MJ/ kg of algal biomass
Sturm and Lamer, 2011	15 MJ/ kg of algal biomass
Sevigné-Itoiz et al., 2012	7.1 MJ/ kg of algal biomass

Table 6.2: literature input of energy demand for centrifugation. These inputs are compared to the amount used for this work

Figure 6.1 and Table 6.1 show that lime flocculation could be an interesting option. In fact, both aluminum and lime flocculation can be used for harvesting. They have similar impacts. Lime flocculation has the lowest impacts for AP, EP, carcinogens and non carcinogens for all scenarios. Non renewable energy consumption, GWP, ODP and POCP are worse than those of aluminum flocculation. Lime and aluminum flocculation appear interchangeable in fact the energy demand for aluminum and lime flocculation is the same (0.54 MJ/ kg of algal biomass) but the amount of lime (0.449 kg/ kg of algal biomass) is higher than that of aluminum sulphate (0.105 kg/ kg of algal biomass). Lime is less toxic than aluminum sulphate. On the other hand, the use of lime in water can form precipitate such as $CaCO_3$ that has to be disposed after algal harvesting whereas aluminum sulphate forms aluminum hydroxide which removes algal cells by water without any precipitates (Demirbas et al., 2010). Aluminum sulphate has a pH range (4.0-7.0) lower than lime (9.0-11). For this reason, pH adjustments for aluminum sulphate are easily achievable (Brentner et al., 2011). Therefore, aluminum flocculation is used more than lime flocculation even if one limitation of aluminum flocculation is about aluminum sulphate toxicity, which can affect anaerobic digestion performance. In fact, the main compound of aluminum flocculation is aluminum hydroxide, forming agglomerate with algal biomass. The residual biomass used for anaerobic digestion contains this toxic compound for methanogens (Pedroni et al., 2001). Bio-flocculation of microalgae could be an interesting solution even if some aspects need to be improved. Nutrients stress, environmental conditions and the choice of algal strains play an important role for the development of bio-flocculation, requiring low costs and energy demand (Pedroni et al., 2001).

In this study, the energy demand for flocculation (0.54 MJ/ kg of algal biomass) is similar to those required in the works of Stephenson and coauthors (2010) and of Razon and Tan (2011), as shown in Table 6.3. Lardon and coauthors (2009) and Soratana and coauthors (2012) have coupled flocculation with drying phase and this could explain why energy demands for flocculation are higher than those of Brentner and coauthors (2011), Stephenson and coauthors (2010) and of Razon and Tan (2011).

Authors	**Energy demand for flocculation**
Lardon et al., 2009	7.20 MJ/ kg of algal biomass
Stephenson et al., 2010	0.50 MJ/ kg of algal biomass
Sturm and Lamer, 2011	3.00 MJ/ kg of algal biomass
Razon and Tan, 2011	0.96 MJ/ kg of algal biomass
Soratana et al., 2012	6.84 MJ/ kg of algal biomass

Table 6.3: literature input of energy demand for flocculation. These inputs are compared to the amount used for this work

As far as extraction phase concerns, the scenarios assuming extraction with hexane correspond to the first three columns of Figure 6.1; and the scenarios assuming extraction with sCO_2 correspond to the second three columns for both freshwater and wastewater scenarios. As it is possible to observe, sCO_2 extraction has lower impacts than hexane extraction in all impact categories. Due to the avoided drying, sCO_2 extraction has a low energy demand. For this reason, these results are expected.

All works analyzed in section 1.7 do not mention sCO_2 extraction like an oil extraction method. In the works of Lardon and coauthors (2009) and Xu and coauthors (2011), wet extraction is carried out. Xu and coauthors (2011) performed a wet extraction with methanol while Lardon and coauthors (2009) mentioned a wet extraction with hexane. This situation does not allow the comparison of this work with the previous works.

As it is possible to observe, Figure 6.2 and Table 6.4 show LCIA results of all scenarios when waste CO_2 is used. All data are normalized to basic scenario (cultivation in freshwater, aluminum flocculation and hexane extraction) and they are presented in percentage. In Figure 6.2, EP is not included because differences between basic scenario and "wastewater scenarios" correspond to two or more orders of magnitude, as shown in Table 6.4. Therefore, should be impossible to show the EP values in the figure.

The use of waste CO_2 implies that the difference between "freshwater and wastewater scenarios" varies for each category. This difference corresponds to 50% in aquatic AP; 65% for GWP and terrestrial AP; 75% in HT and 40% in ODP, POCP and non renewable energy consumption. Except to GWP, the trend of "wastewater scenarios" is the same of those of the "freshwater scenarios". For this reason, the following considerations are referred to scenarios with freshwater but they are qualitative the same for "wastewater scenarios".

Table 6.4 shows impacts higher than basic scenario in red and impacts lower than basic scenario in green.

As it possible to observe, waste CO_2 does not improve environmental performance of centrifugation. This technology is the worst option, impacting more than basic scenario in aquatic and terrestrial AP, GWP, non renewable energy consumption, ODP and POCP. On the other hand, centrifugation shows lower impact than aluminum flocculation in carcinogens and non carcinogens. As far as lime flocculation regards, differences between aluminum flocculation are not relevant in aquatic AP, carcinogens and non carcinogens. In terrestrial AP, lime flocculation has the same impact of aluminum flocculation but it impacts more than basic scenario in ODP, GWP, POCP and non renewable energy consumption. As stated previously, from the environmental point of view, lime and aluminum flocculation could be used indifferently but for a high pH adjustment and precipitates formation, lime is not as used as aluminum sulphate.

Figure 6.2 shows the scenarios assuming extraction with hexane in the first three columns; and the scenarios assuming extraction with sCO_2 in the second three columns for both “freshwater and wastewater scenarios”.

Concerning to extraction methods, it is possible to observe that in carcinogens and non carcinogens, sCO_2 extraction has lower impacts than hexane extraction and they differ significantly by about 40% on average. Since sCO_2 extraction does not require thermal energy for drying phase, it impacts significantly less than basic scenario in non renewable energy consumption, GWP, AP, ODP and POCP.

In “wastewater scenarios”, in comparison to basic scenario, GWP can avoid impacts, corresponding to negative columns. A GHG emissions saving is only reached by the scenario which considers both aluminum flocculation and sCO_2 extraction. The waste CO_2 can improve significantly impacts for “wastewater scenarios”, reaching also avoided impacts when wet extraction is carried out.

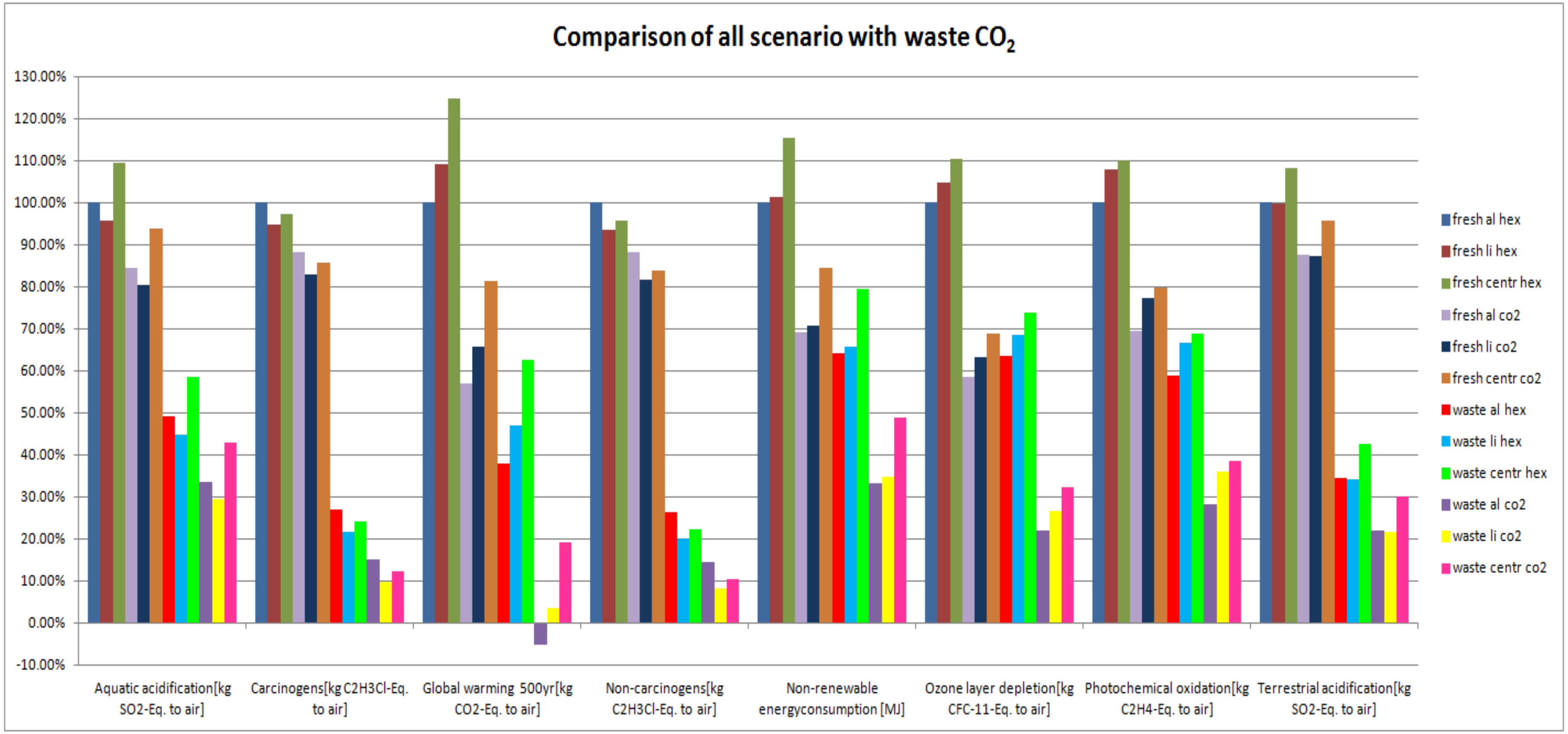
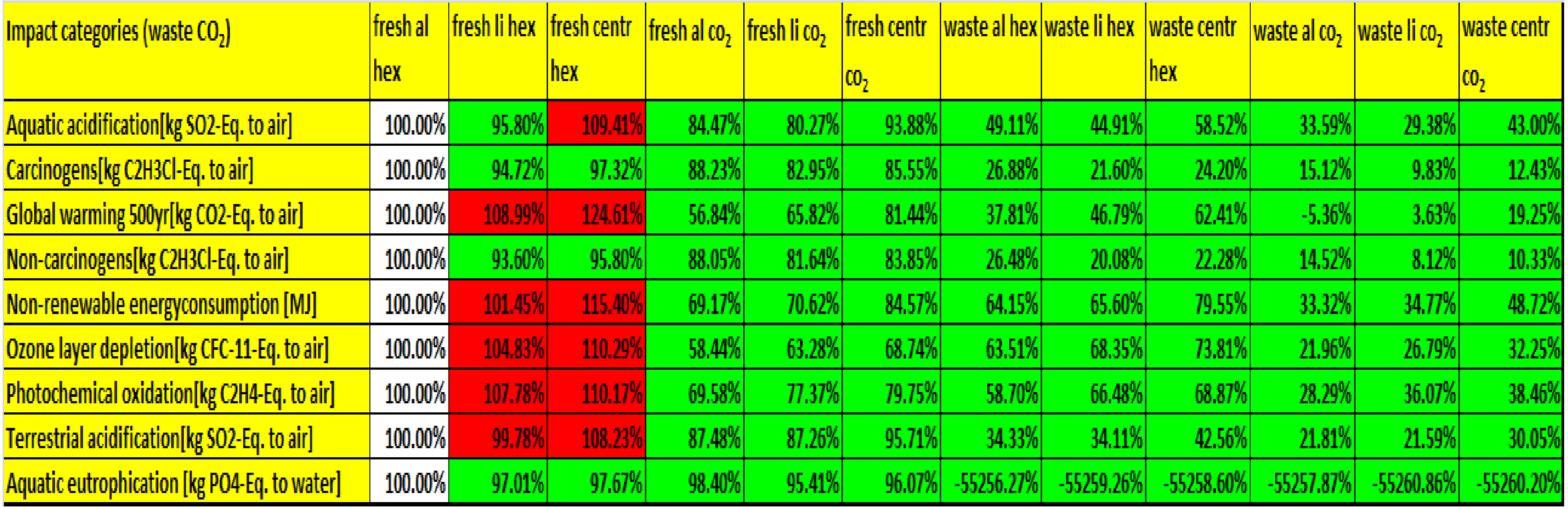


Figure 6.2: "freshwater, aluminum flocculation and hexane extraction" is the basic scenario (100%). Others scenarios are compared to it. EP is not included because the difference between basic scenario and scenarios with the use of wastewater is more than one order of magnitude.



Impact categories (waste CO$_2$)	fresh al hex	fresh li hex	fresh centr hex	fresh al co$_2$	fresh li co$_2$	fresh centr co$_2$	waste al hex	waste li hex	waste centr hex	waste al co$_2$	waste li co$_2$	waste centr co$_2$
Aquatic acidification[kg SO2-Eq. to air]	100.00%	95.80%	109.41%	84.47%	80.27%	93.88%	49.11%	44.91%	58.52%	33.59%	29.38%	43.00%
Carcinogens[kg C2H3Cl-Eq. to air]	100.00%	94.72%	97.32%	88.23%	82.95%	85.55%	26.88%	21.60%	24.20%	15.12%	9.83%	12.43%
Global warming 500yr[kg CO2-Eq. to air]	100.00%	108.99%	124.61%	56.84%	65.82%	81.44%	37.81%	46.79%	62.41%	-5.36%	3.63%	19.25%
Non-carcinogens[kg C2H3Cl-Eq. to air]	100.00%	93.60%	95.80%	88.05%	81.64%	83.85%	26.48%	20.08%	22.28%	14.52%	8.12%	10.33%
Non-renewable energyconsumption [MJ]	100.00%	101.45%	115.40%	69.17%	70.62%	84.57%	64.15%	65.60%	79.55%	33.32%	34.77%	48.72%
Ozone layer depletion[kg CFC-11-Eq. to air]	100.00%	104.83%	110.29%	58.44%	63.28%	68.74%	63.51%	68.35%	73.81%	21.96%	26.79%	32.25%
Photochemical oxidation[kg C2H4-Eq. to air]	100.00%	107.78%	110.17%	69.58%	77.37%	79.75%	58.70%	66.48%	68.87%	28.29%	36.07%	38.46%
Terrestrial acidification[kg SO2-Eq. to air]	100.00%	99.78%	108.23%	87.48%	87.26%	95.71%	34.33%	34.11%	42.56%	21.81%	21.59%	30.05%
Aquatic eutrophication [kg PO4-Eq. to water]	100.00%	97.01%	97.67%	98.40%	95.41%	96.07%	-55256.27%	-55259.26%	-55258.60%	-55257.87%	-55260.86%	-55260.20%

Table 6.4: normalization of LCIA results. These results are carried out with the use of waste CO$_2$. “Freshwater, aluminum flocculation and hexane extraction” is basic scenario. For this reason, basic scenario is set to 1. Others scenarios are normalized respect to “freshwater, aluminum flocculation and hexane extraction”. Scenarios worse than he basic are in red whereas scenarios better than basic are in green. In EP, the scenarios with the use of wastewater shows a difference of three orders of magnitude compared with the basic one.

Summering results of both synthetic and waste CO_2 use, some issues has to be highlighted:

1. Except for human toxicity and eutrophication, **centrifugation is the worst option** in all 24 scenarios analyzed. Obviously, centrifugation in "wastewater scenarios" impacts less than basic scenario but also in this case, it is worse than flocculation.

From the environmental point of view, aluminum and lime flocculation could be interchangeable because they show similar impacts.

2. **sCO_2 extraction has better environmental performance than hexane extraction** in all scenarios analyzed.

3. Obviously, "**wastewater scenarios" have better environmental performance than corresponding scenario using freshwater.** GWP and EP show lower impacts than basic scenario, also reaching avoided impacts. These results agree with the analysis of LCIA results performed in section 5.1. On the other hand, it is important to highlight that currently the use of wastewater as algae cultivation water is not developed on commercial scale but only on pilot plants. In Spain "All-gas" project uses wastewater treatment plant in order to use water and nutrients such as nitrogen and phosphorus for stimulating algal growth. This project aims to demonstrate the sustainable large-scale production of biofuels based on the low-cost cultivation of microalgae (http://www.all-gas.eu/Pages/default.aspx). Another important aspect to be highlighted is that the use of wastewater in PBR needs to be enhanced. In fact the presence of effluents in the cultivation water does not allow the light penetration. Hence, a water clarification pretreatment should be necessary in order to reduce the presence of these effluents and the organic load even if this process requires a large amount of water. Moreover the sewage in the water can reduce the material resistance of PBR (glass, polycarbonate, LDPE). In order to improve wastewater cultivation in PBR, wear-resistant materials must be used.

4. **The use of waste CO_2 allows negative GHG emissions** only for "aluminum flocculation and sCO_2 extraction" while synthetic CO_2 does not show GHG emissions saving. Obviously, the use of waste CO_2 coupled with wastewater can decrease impact for all categories but these two technologies need yet to be developed on large scale. As far as waste CO_2 use concerns, flue gas transfer from a power plant to PBR and CO_2 loss are the main problems to be solved. Due to the large energy demand for

pumping a flue gas, the distance from the power plant to PBR limits this transfer (Pedroni et al., 2001). In order to avoid a CO_2 loss, the transfer efficiency should correspond to 80%-90% (Pedroni et al., 2001). In Alicante, the first industrial pilot plant using CO_2 captured from a cement industry has been operating since 2010. The project aims to produce bio-petrol from microalgae. The experimental results that, during the first phase of processing, high-value nutrients can also be extracted from the biomass (http://www.biopetroleo.com/).

5. Our study indicates that in order to develop a biodiesel production system, the option assuming **“cultivation with waste CO_2 and wastewater, flocculation with aluminum sulphate and extraction with sCO_2” could be interesting** since it could reduce environmental impacts avoiding GHG emissions respect to “aluminum flocculation and hexane extraction”. But this scenario needs to exceed the limits mentioned above about the use of waste CO_2 and wastewater for algal cultivation.

7 SENSITIVITY ANALYSIS

The aim of this section is to identify the most important parameters to achieve the sustainability of the biodiesel production from algae.

A sensitivity analysis is carried out. It identifies and focuses on key data and assumptions that have most influence on the results. The main assumptions are described in section 4.2.1. Among them, conversion efficiency, the ratio of nitrogen and phosphorus supplies for the algal growth and Danish electricity mix have already been calculated by real, reliable and consistent data. On the other hand, lipid content and extraction efficiency can vary, depending on the conditions in which the case study has been carried out.

For this reason, this sensitivity analysis considers the following parameters:

1. lipid content;
2. extraction efficiency.

According to literature analysis, the extraction efficiency varies in a range from 0.91(Khoo et al., 2010) to 0.95 (Brentner et al., 2011).

In the Basic Case, the lowest value (0.91) is chosen for both hexane and sCO_2 extraction (see section 4.2.1) whereas Case 1 is performed with the highest value for the extraction efficiency corresponding to 0.95. Table 7.1 illustrates the different cases analyzed.

Lipid content is the other important parameter for biodiesel production. For this reason, a literature analysis about lipid content for *Nannochloropsis* is done. Rodolfi and coauthors (2009) assessed that *Nannochloropsis* can accumulate lipid from 29.2% to 60.9% of dry weight of biomass

Batan and coauthors (2010) and Jorquera and coauthors (2010) stated that *Nannochloropsis* can reach 60% in lipid content.

Khoo and coauthors (2011) performed sensitivity analysis varying lipid content from 25% to 35% and 45%. They observed that the increasing is only achieved when *Nannochloropsis* cultivation reaches nitrogen stress conditions. They also demonstrated that the increase of lipid content by about 10% and 20% decreases the energy consumption by about 4% and 6%, respectively.

Razon and Tan (2011) carried out a sensitivity analysis about lipid content of *Nannochloropsis,* varying its lipid content from 30.1% to 35.7 (Rodolfi et al., 2009). They observed that an increase of lipid content decreases the net energy demand (MJ) for the production of 1 kg of methyl esters.

Basing on this literature analysis and these considerations, an experiment on sensitivity related to lipid content was carried out. Therefore, Case 2 considers the highest lipid content (60%) for *Nannochloropsis* without changing the nitrogen supply.

Instead, in the Basic Case, lipid content is assumed to be the lowest value corresponding to 29% (see section 4.2.1).

The following sections analyze Case 1 and Case 2. The most relevant categories are GWP and non renewable energy consumption.

For this reason, they are summarized in Table 7.1. In Case 1 and Case 2, “freshwater and wastewater scenarios” are investigated with the use of synthetic and waste CO_2 alternatively.

Parameter	**Basic Case**	**Case 1**	**Case 2**
Synthetic CO_2	**Synthetic CO_2**	**Synthetic CO_2**	**Synthetic CO_2**
Extraction efficiency	0.91	0.95	0.91
Lipid content	29%	29%	60%
CO_2 saving	no	no	no
Energy saving	no	no	no
Parameter	**Basic Case**	**Case 1**	**Case 2**
Waste CO_2	**Waste CO_2**	**Waste CO_2**	**Waste CO_2**
Extraction efficiency	0.91	0.95	0.91
Lipid content	29%	29%	60%
CO_2 saving	yes only for wastewater scenario with aluminum flocculation and sCO_2 extraction	yes only for wastewater scenario with aluminum flocculation and sCO_2 extraction	yes only for wastewater scenario with aluminum flocculation and sCO_2 extraction
Energy saving	no	no	no

Table 7.1: summary of different cases performed in sensitivity analysis. Basic case is set to scenario 1 (section 3.2.5). Synthetic and waste CO_2 are assessed.

7.1 Case 1: the increase of extraction efficiency from 0.91 to 0.95

Case 1 describes a variation of extraction efficiency from 0.91 to 0.95. The use of synthetic and waste CO_2 are analyzed, respectively. Figure 7.1 and Figure 7.2 show GWP and non renewable energy consumption in the case that synthetic CO_2 is used. For all scenarios, in both categories, algal biodiesel is worse than diesel and also the environmental performance of hexane extraction does not differ significantly to that of sCO_2 extraction. Compared to the Basic Case, the GHG emissions decrease but not significantly.

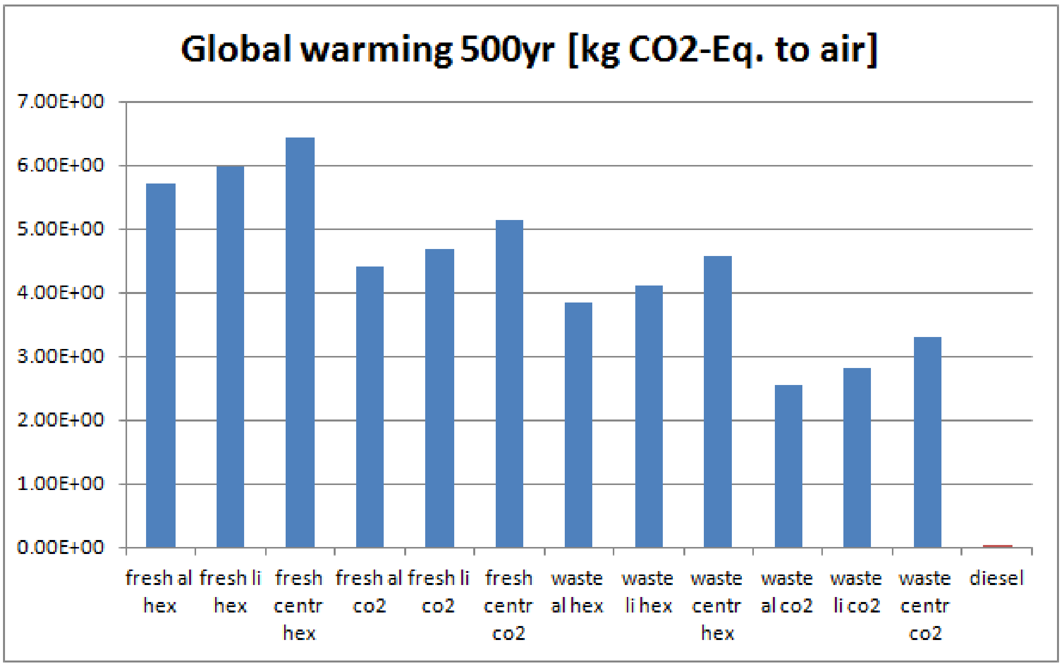


Figure 7.1: GWPs for Case 1 when synthetic CO_2 is used. Comparison between algal biodiesel and diesel from fossil fuels (red column)

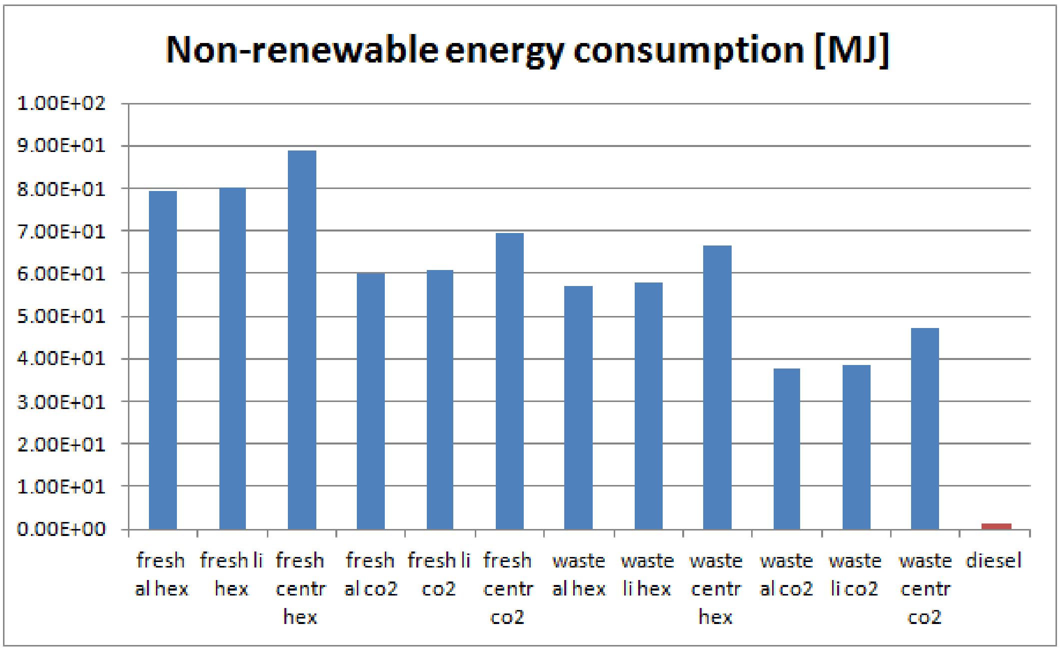
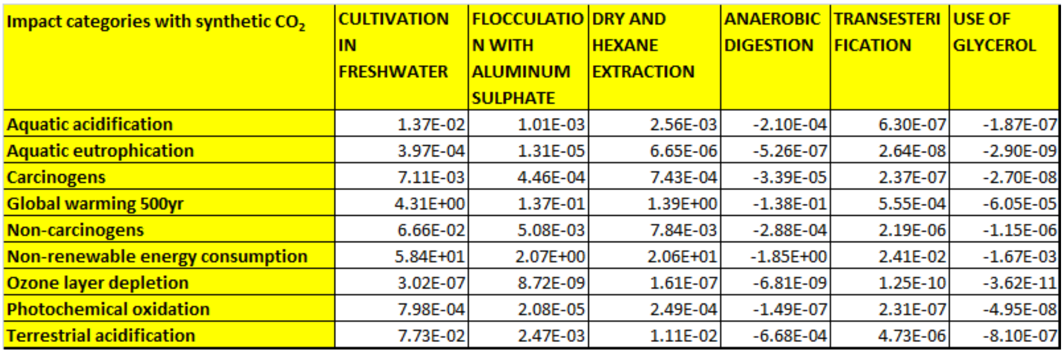


Figure 7.2: non renewable energy consumption for Case 1 when synthetic CO_2 is used. Comparison between algal biodiesel and diesel from fossil fuels (red column)

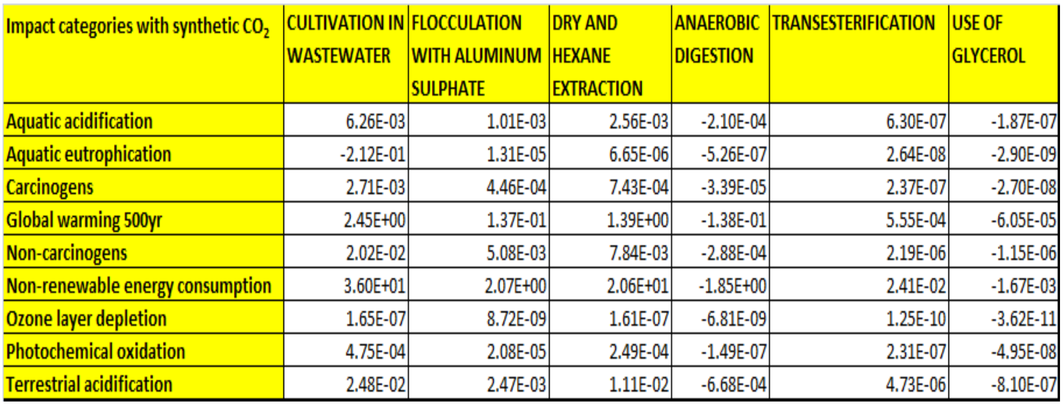
Table 7.2 shows the total contributions of each process to all impact categories in case that freshwater and synthetic CO_2 are considered. Compared to Table 5.4, cultivation phase, aluminum flocculation drying phase coupled with hexane extraction decrease their total contributions to all categories. On the other hand, anaerobic digestion has lower avoided impacts than those of the Basic Case. Despite the decrease of total contribution of each phase, the relative contributions of each process to all impact categories are the same that those of Basic Case (Figure 5.17).



Impact categories with synthetic CO_2	CULTIVATION IN FRESHWATER	FLOCCULATION WITH ALUMINUM SULPHATE	DRY AND HEXANE EXTRACTION	ANAEROBIC DIGESTION	TRANSESTERIFICATION	USE OF GLYCEROL
Aquatic acidification	1.37E-02	1.01E-03	2.56E-03	-2.10E-04	6.30E-07	-1.87E-07
Aquatic eutrophication	3.97E-04	1.31E-05	6.65E-06	-5.26E-07	2.64E-08	-2.90E-09
Carcinogens	7.11E-03	4.46E-04	7.43E-04	-3.39E-05	2.37E-07	-2.70E-08
Global warming 500yr	4.31E+00	1.37E-01	1.39E+00	-1.38E-01	5.55E-04	-6.05E-05
Non-carcinogens	6.66E-02	5.08E-03	7.84E-03	-2.88E-04	2.19E-06	-1.15E-06
Non-renewable energy consumption	5.84E+01	2.07E+00	2.06E+01	-1.85E+00	2.41E-02	-1.67E-03
Ozone layer depletion	3.02E-07	8.72E-09	1.61E-07	-6.81E-09	1.25E-10	-3.62E-11
Photochemical oxidation	7.98E-04	2.08E-05	2.49E-04	-1.49E-07	2.31E-07	-4.95E-08
Terrestrial acidification	7.73E-02	2.47E-03	1.11E-02	-6.68E-04	4.73E-06	-8.10E-07

Table 7.2: Case 1: the total contribution of each process to all categories when synthetic CO_2 and freshwater are considered

Table 7.3 illustrates the total contributions of each phase to all categories when synthetic CO_2 and wastewater for algae cultivation are assessed. Also in this case, the increase of extraction efficiency decreases not significantly the contributions of all processes in comparison to the Basic Case (Table 5.6). The relative contributions of each process are the same of those of the Basic Case (Figure 5.19)



Impact categories with synthetic CO_2	CULTIVATION IN WASTEWATER	FLOCCULATION WITH ALUMINUM SULPHATE	DRY AND HEXANE EXTRACTION	ANAEROBIC DIGESTION	TRANSESTERIFICATION	USE OF GLYCEROL
Aquatic acidification	6.26E-03	1.01E-03	2.56E-03	-2.10E-04	6.30E-07	-1.87E-07
Aquatic eutrophication	-2.12E-01	1.31E-05	6.65E-06	-5.26E-07	2.64E-08	-2.90E-09
Carcinogens	2.71E-03	4.46E-04	7.43E-04	-3.39E-05	2.37E-07	-2.70E-08
Global warming 500yr	2.45E+00	1.37E-01	1.39E+00	-1.38E-01	5.55E-04	-6.05E-05
Non-carcinogens	2.02E-02	5.08E-03	7.84E-03	-2.88E-04	2.19E-06	-1.15E-06
Non-renewable energy consumption	3.60E+01	2.07E+00	2.06E+01	-1.85E+00	2.41E-02	-1.67E-03
Ozone layer depletion	1.65E-07	8.72E-09	1.61E-07	-6.81E-09	1.25E-10	-3.62E-11
Photochemical oxidation	4.75E-04	2.08E-05	2.49E-04	-1.49E-07	2.31E-07	-4.95E-08
Terrestrial acidification	2.48E-02	2.47E-03	1.11E-02	-6.68E-04	4.73E-06	-8.10E-07

Table 7.3: Case 1: the total contribution of each process to all categories when synthetic CO_2 and wastewater are considered

Figure 7.3 and Figure 7.4 show GWP and non renewable energy consumption when waste CO_2 is used. Figure 7.3 shows that all scenarios are worse than diesel excluding “wastewater, aluminum flocculation and sCO_2 extraction”. This scenario avoids GHG emissions, highlighting their better environmental performance than that of the diesel. Figure 7.4 shows that non renewable energy consumption for algal biodiesel is higher than diesel for all scenarios. Compared to the Basic Case, the decrease of GHG emissions and energy demand is not significantly important.

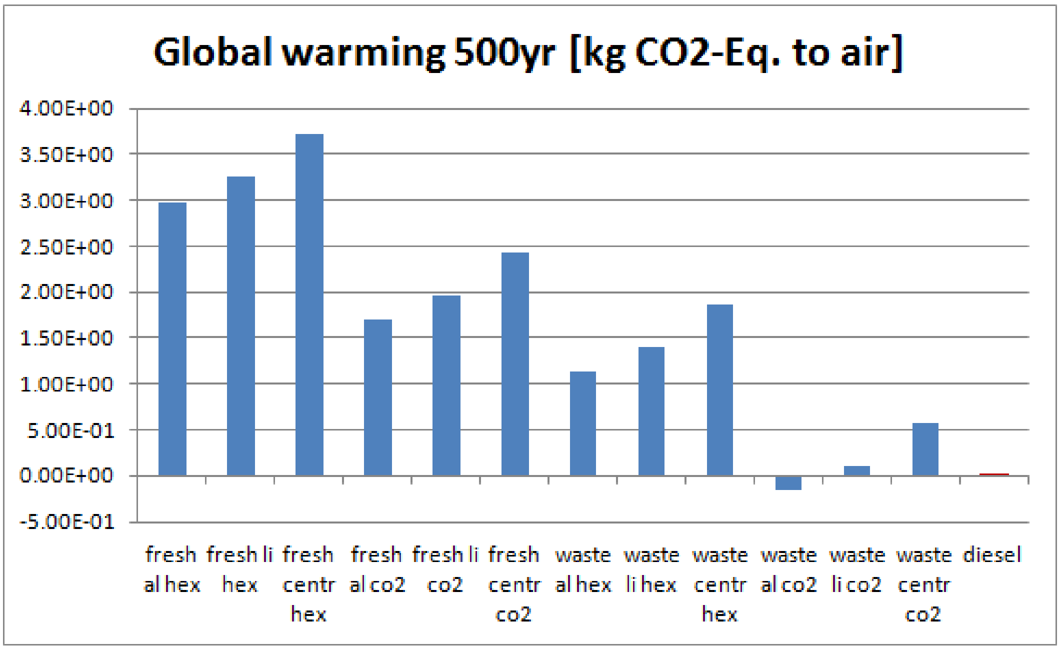


Figure 7.3: GWPs for Case 1 when waste CO_2 is used. Comparison between algal biodiesel and diesel from fossil fuels (red column)

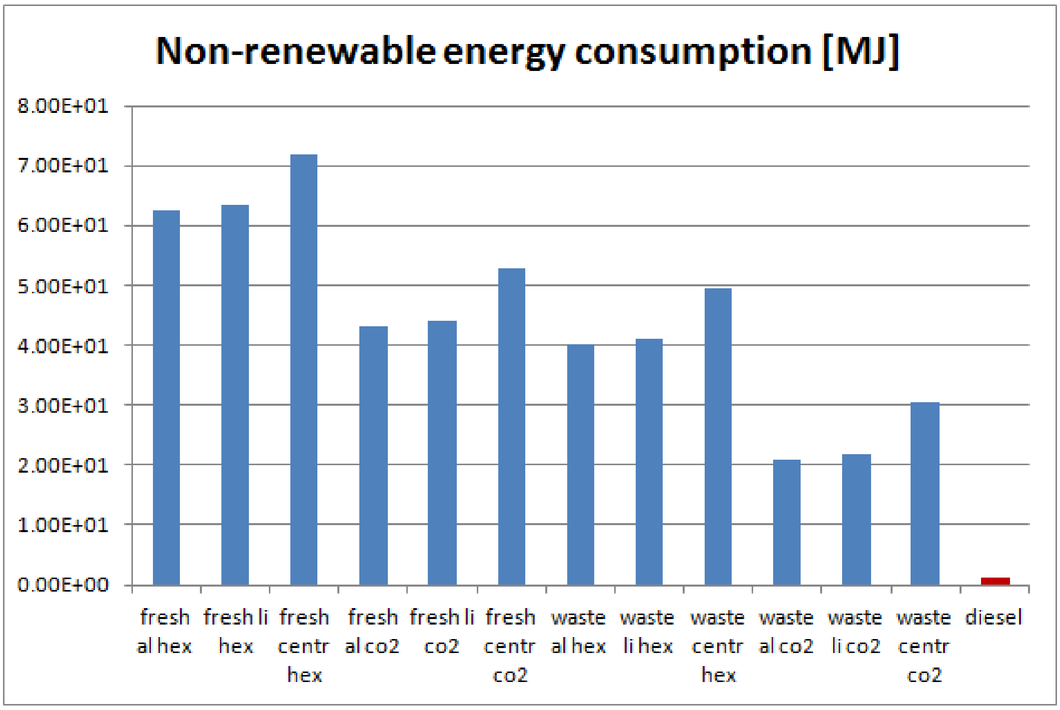


Figure 7.4: non renewable energy consumption for Case 1 when waste CO_2 is used. Comparison between algal biodiesel and diesel from fossil fuels (red column)

Table 7.4 and Table 7.5 show the total contributions of each phase to all categories when waste CO_2 replaces synthetic CO_2. Table 7.4 analyses the cultivation in freshwater whereas in Table 7.5 cultivation phase is carried out for “wastewater scenario”. Compared to Table 5.5 and Table 5.7, all processes have lower impacts than Basic Case but their decrease is not relevant for algal biodiesel production. Also in this case, the less impacting scenario uses wastewater and waste CO_2 and its difference with Basic Case is not significative, corresponding to the same order of magnitude. Obviously, also in this case, the relative contributions of each phase are quantitatively the same of the ones for Basic Case (Figure 5.18 and Figure 5.20).

Impact categories with waste CO_2	CULTIVATION IN FRESHWATER	FLOCCULATION WITH ALUMINUM SULPHATE	DRY AND HEXANE EXTRACTION	ANAEROBIC DIGESTION	TRANSESTERIFICATION	USE OF GLYCEROL
Aquatic acidification	1.13E-02	1.01E-03	2.56E-03	-2.10E-04	6.30E-07	-1.87E-07
Aquatic eutrophication	3.65E-04	1.31E-05	6.65E-06	-5.26E-07	2.64E-08	-2.90E-09
Carcinogens	4.87E-03	4.46E-04	7.43E-04	-3.39E-05	2.37E-07	-2.70E-08
Global warming 500yr	1.59E+00	1.37E-01	1.39E+00	-1.38E-01	5.55E-04	-6.05E-05
Non-carcinogens	5.05E-02	5.08E-03	7.84E-03	-2.88E-04	2.19E-06	-1.15E-06
Non-renewable energy consumption	4.15E+01	2.07E+00	2.06E+01	-1.85E+00	2.41E-02	-1.67E-03
Ozone layer depletion	2.11E-07	8.72E-09	1.61E-07	-6.81E-09	1.25E-10	-3.62E-11
Photochemical oxidation	5.10E-04	2.08E-05	2.49E-04	-1.49E-07	2.31E-07	-4.95E-08
Terrestrial acidification	6.70E-02	2.47E-03	1.11E-02	-6.68E-04	4.73E-06	-8.10E-07

Table 7.4: Case 1: the total contribution of each process to all categories when waste CO_2 and freshwater are considered

Impact categories with waste CO_2	CULTIVATION IN WASTEWATER	FLOCCULATION WITH ALUMINUM SULPHATE	DRY AND HEXANE EXTRACTION	ANAEROBIC DIGESTION	TRANSESTERIFICATION	USE OF GLYCEROL
Aquatic acidification	3.82E-03	1.01E-03	2.56E-03	-2.10E-04	6.30E-07	-1.87E-07
Aquatic eutrophication	-2.13E-01	1.31E-05	6.65E-06	-5.26E-07	2.64E-08	-2.90E-09
Carcinogens	4.64E-04	4.46E-04	7.43E-04	-3.39E-05	2.37E-07	-2.70E-08
Global warming 500yr	-2.66E-01	1.37E-01	1.39E+00	-1.38E-01	5.55E-04	-6.05E-05
Non-carcinogens	4.09E-03	5.08E-03	7.84E-03	-2.88E-04	2.19E-06	-1.15E-06
Non-renewable energy consumption	1.92E+01	2.07E+00	2.06E+01	-1.85E+00	2.41E-02	-1.67E-03
Ozone layer depletion	7.47E-08	8.72E-09	1.61E-07	-6.81E-09	1.25E-10	-3.62E-11
Photochemical oxidation	1.88E-04	2.08E-05	2.49E-04	-1.49E-07	2.31E-07	-4.95E-08
Terrestrial acidification	1.46E-02	2.47E-03	1.11E-02	-6.68E-04	4.73E-06	-8.10E-07

Table 7.5: Case 1: the total contribution of each process to all categories when waste CO_2 and wastewater are considered

Some main issues can be discussed as follows:

1. When extraction efficiency increases, GWP and non renewable energy consumption have lower impacts than Basic Case. However, the decrease of GHG emissions and energy consumption are in the same order of magnitude of the one for the Basic Case. Therefore, **extraction efficiency does not significantly affect the environmental performance of biodiesel**.
2. All processes decrease their total contributions to all impact categories. This means that AD decreases avoided impacts to all categories analyzed. In fact, **the increase of the extraction efficiency decreases the quantity of the residual biomass** used for anaerobic digestion. In all

scenarios analyzed, the relative contributions of each process to all categories are quantitatively the same of those of the Basic Case.

3. Only when wastewater and waste CO_2 are used, the use of wastewater coupled with **aluminum flocculation and sCO_2 extraction makes the environmental performance of biodiesel better** than diesel.

7.2 Case 2: the increase of lipid content from 29% to 60%

Case 2 is performed by an increase of lipid content from 29% to 60%. GWP and non renewable energy consumption consider both synthetic and waste CO_2.

Figure 7.5 shows GWPs for algal biodiesel when synthetic CO_2 is used. All scenarios have GWP higher than the one of the diesel. Compared to Figure 5.1, the increase of lipid content decreases GHG emissions by about 50% but the biodiesel production does not significantly improve its environmental performance. This is due to the large amount of heat required for drying phase and also for the large CO_2 demand for algal growth. In addition, a few case studies have investigated the increase of lipid content in laboratory (Pittman et al., 2011). They highlight that the increase of lipid content could decrease the biomass productivity and it is necessary to find a lipid content such that biomass productivity is not too low.

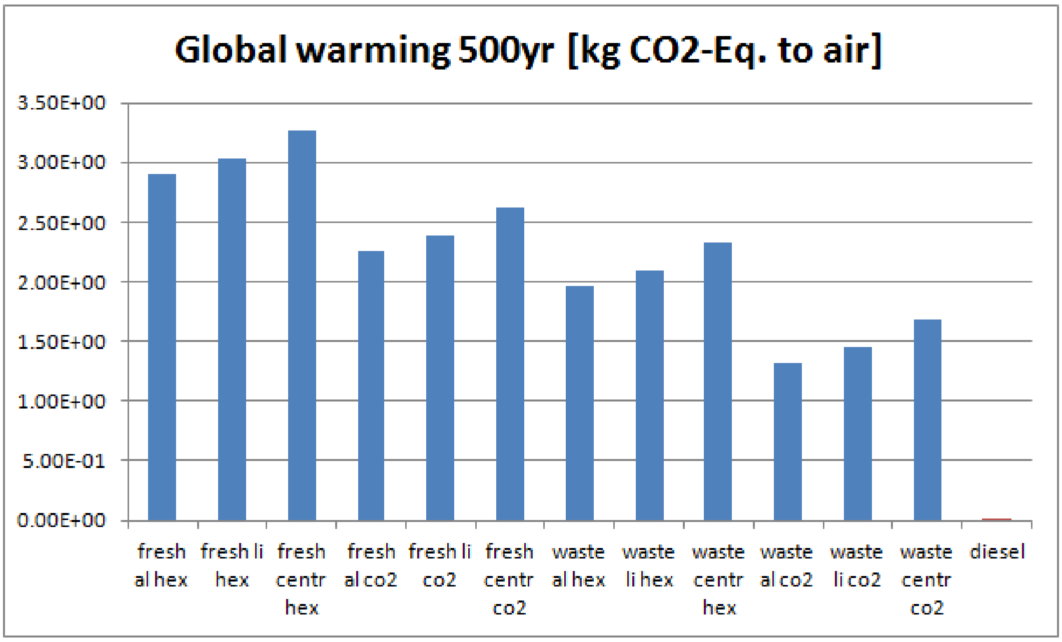


Figure 7.5: Case 2. GWPs when synthetic CO_2 is used. Comparison between algal biodiesel and diesel from fossil fuels (red column)

Figure 7.6 illustrates non renewable energy consumption for algal biodiesel and diesel (in red). All scenarios show worse environmental performance than diesel even if the energy demand in Case 2 decreases by about 50% compared to Figure 5.3.

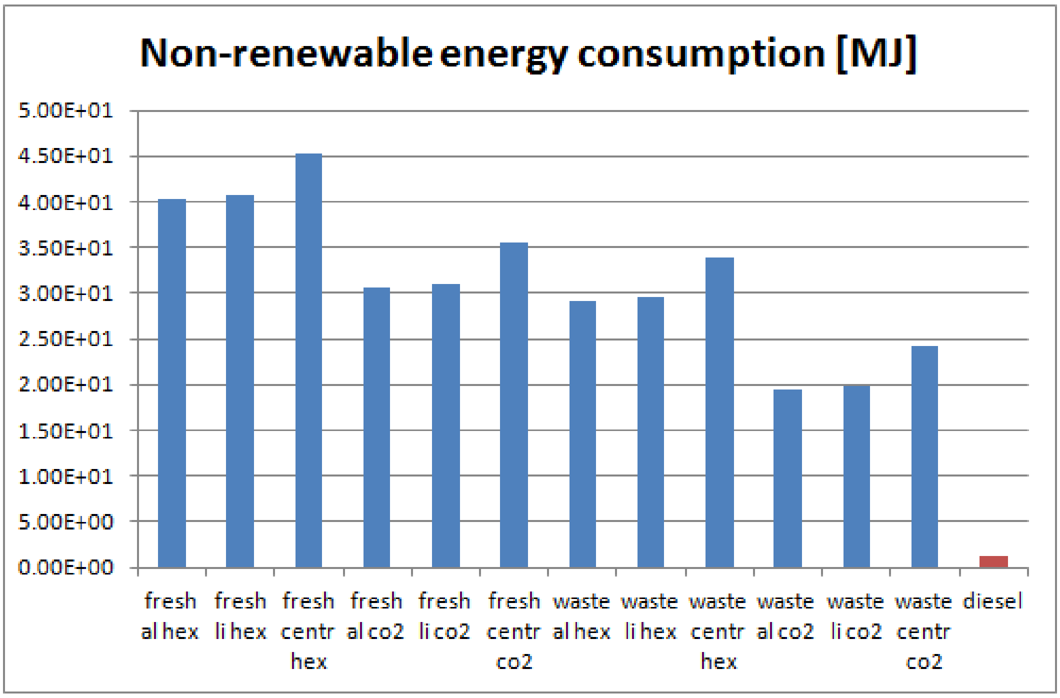
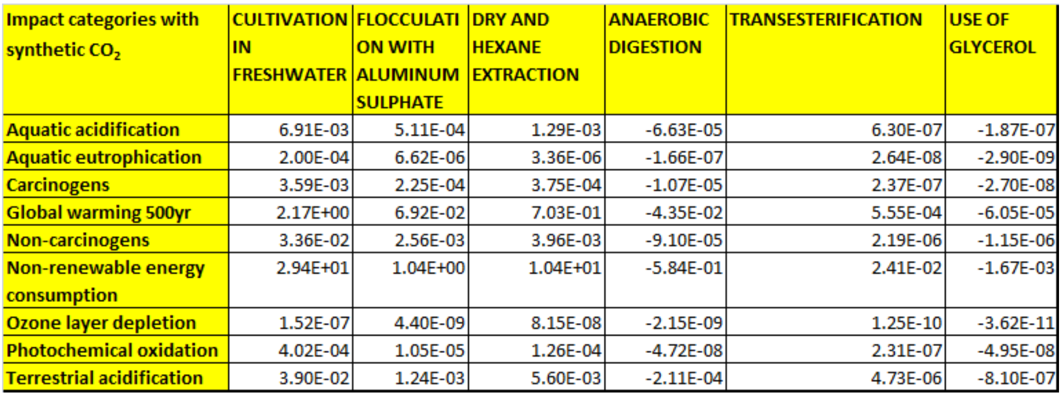


Figure 7.6: Case 2. Non renewable energy consumption when synthetic CO_2 is used. Comparison between algal biodiesel and diesel from fossil fuels (red column)

The total contributions of each process to all categories are shown in Table 7.6 and Table 7.7 when synthetic CO_2 is used and "freshwater and wastewater scenarios" are respectively analyzed. Compared to the Basic Case (Table 5.4 and Table 5.6), cultivation, flocculation, drying phase with hexane extraction and anaerobic digestion decrease their contributions. The increase of lipid content also decreases the amount of algal biomass produced. For this reason, the increase of lipid content decreases these contributions by about 50% but the algal biodiesel sustainability has not been reached, yet.

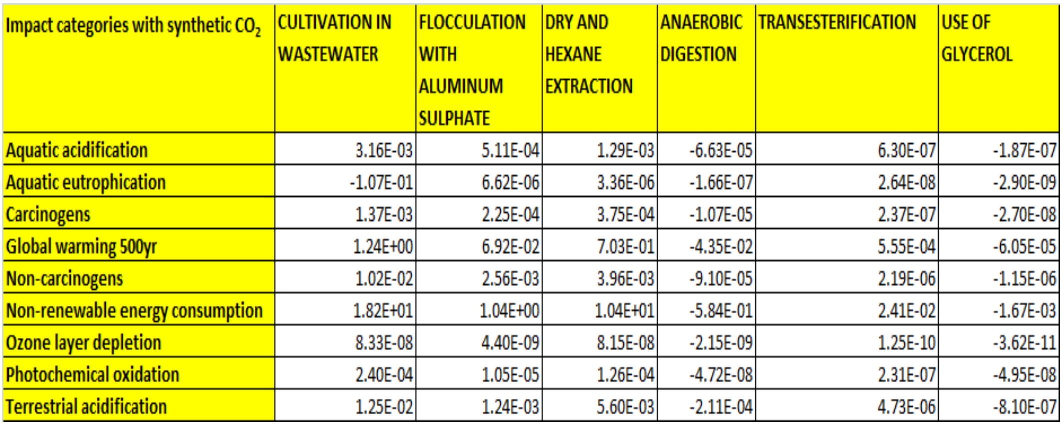
The contributions of transesterification and the glycerol use are negligible. In addition, anaerobic digestion has lower avoided impacts than those of the Basic Case.

In this case, the relative contributions of each process to all impact categories are the same that those of Basic Case (Figure 5.17 and Figure 5.19).



Impact categories with synthetic CO_2	CULTIVATION IN FRESHWATER	FLOCCULATION WITH ALUMINUM SULPHATE	DRY AND HEXANE EXTRACTION	ANAEROBIC DIGESTION	TRANSESTERIFICATION	USE OF GLYCEROL
Aquatic acidification	6.91E-03	5.11E-04	1.29E-03	-6.63E-05	6.30E-07	-1.87E-07
Aquatic eutrophication	2.00E-04	6.62E-06	3.36E-06	-1.66E-07	2.64E-08	-2.90E-09
Carcinogens	3.59E-03	2.25E-04	3.75E-04	-1.07E-05	2.37E-07	-2.70E-08
Global warming 500yr	2.17E+00	6.92E-02	7.03E-01	-4.35E-02	5.55E-04	-6.05E-05
Non-carcinogens	3.36E-02	2.56E-03	3.96E-03	-9.10E-05	2.19E-06	-1.15E-06
Non-renewable energy consumption	2.94E+01	1.04E+00	1.04E+01	-5.84E-01	2.41E-02	-1.67E-03
Ozone layer depletion	1.52E-07	4.40E-09	8.15E-08	-2.15E-09	1.25E-10	-3.62E-11
Photochemical oxidation	4.02E-04	1.05E-05	1.26E-04	-4.72E-08	2.31E-07	-4.95E-08
Terrestrial acidification	3.90E-02	1.24E-03	5.60E-03	-2.11E-04	4.73E-06	-8.10E-07

Table 7.6: Case 2: the total contribution of each process to all categories when synthetic CO_2 and freshwater are considered



Impact categories with synthetic CO_2	CULTIVATION IN WASTEWATER	FLOCCULATION WITH ALUMINUM SULPHATE	DRY AND HEXANE EXTRACTION	ANAEROBIC DIGESTION	TRANSESTERIFICATION	USE OF GLYCEROL
Aquatic acidification	3.16E-03	5.11E-04	1.29E-03	-6.63E-05	6.30E-07	-1.87E-07
Aquatic eutrophication	-1.07E-01	6.62E-06	3.36E-06	-1.66E-07	2.64E-08	-2.90E-09
Carcinogens	1.37E-03	2.25E-04	3.75E-04	-1.07E-05	2.37E-07	-2.70E-08
Global warming 500yr	1.24E+00	6.92E-02	7.03E-01	-4.35E-02	5.55E-04	-6.05E-05
Non-carcinogens	1.02E-02	2.56E-03	3.96E-03	-9.10E-05	2.19E-06	-1.15E-06
Non-renewable energy consumption	1.82E+01	1.04E+00	1.04E+01	-5.84E-01	2.41E-02	-1.67E-03
Ozone layer depletion	8.33E-08	4.40E-09	8.15E-08	-2.15E-09	1.25E-10	-3.62E-11
Photochemical oxidation	2.40E-04	1.05E-05	1.26E-04	-4.72E-08	2.31E-07	-4.95E-08
Terrestrial acidification	1.25E-02	1.24E-03	5.60E-03	-2.11E-04	4.73E-06	-8.10E-07

Table 7.7: Case 2: the total contribution of each process to all categories when synthetic CO_2 and wastewater are considered

Figure 7.7 and Figure 7.8 illustrate GWP and non renewable energy consumption when waste CO_2 is used.

As it is possible to observe, Figure 7.7 shows that GWP for all scenarios are worse than diesel except to the use of wastewater coupled with aluminum flocculation and sCO_2 extraction. Respect to the Basic Case, the increase of lipid content decreases the GHG emissions by about 50% even if the biodiesel production does not reach lower environmental performance than diesel.

The difference between extraction with hexane and sCO_2 is relevant, corresponding to one order of magnitude. The potential use of waste CO_2 makes “wastewater scenarios” better than those of “freshwater scenarios”, even if they present the same trend. In order to develop the algal biodiesel on industrial scale, wet extraction makes its environmental performance more sustainable than that of diesel.

Figure 7.8 shows that non renewable energy consumption is higher for all scenarios than diesel. Compared to Figure 5.4, also in this case, the increase of lipid content decreases the energy demand for biodiesel production by about 50%. The use of waste CO_2 coupled with the increase of lipid content does not makes biodiesel production better than diesel. The energy demand is always high, producing less energy than that has been consumed.

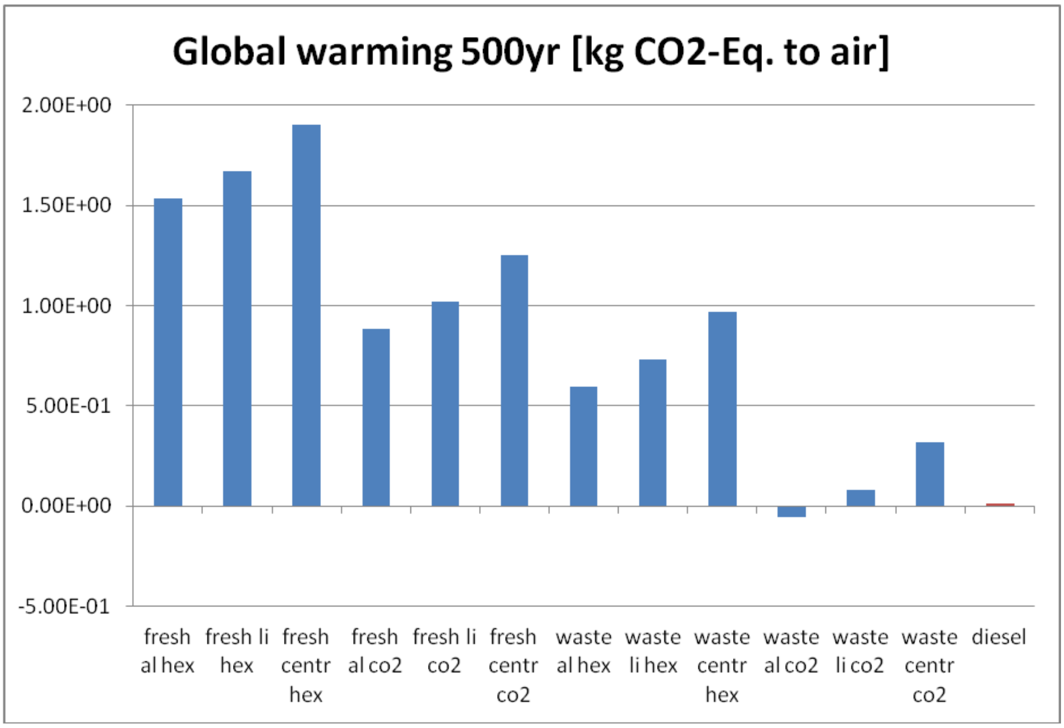


Figure 7.7: GWPs for Case 2 when waste CO_2 is used. Comparison between algal biodiesel and diesel from fossil fuels (red column)

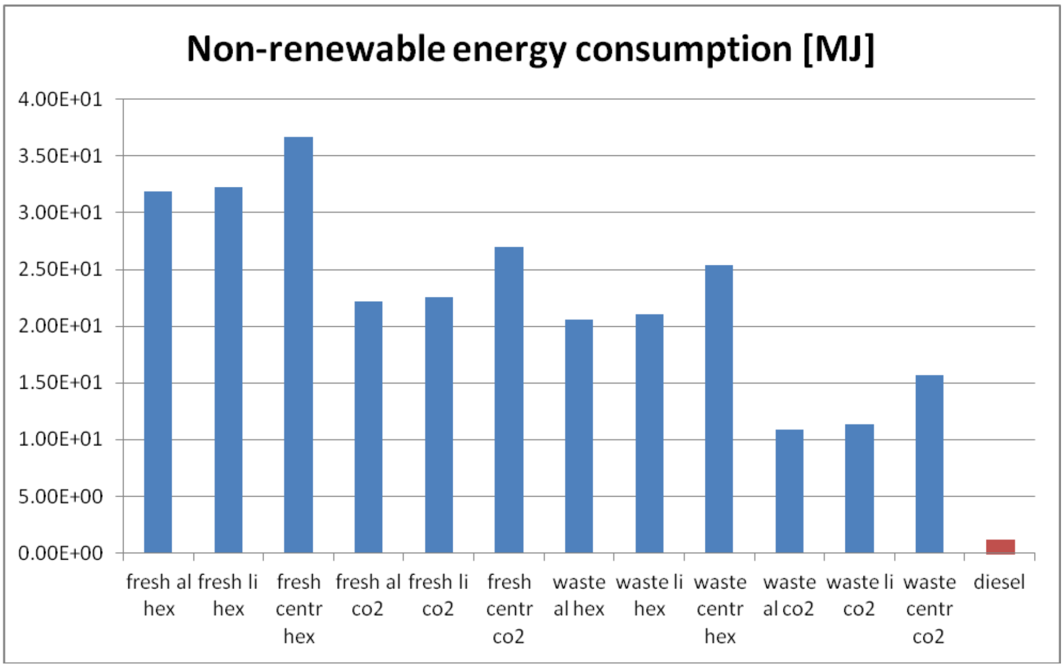


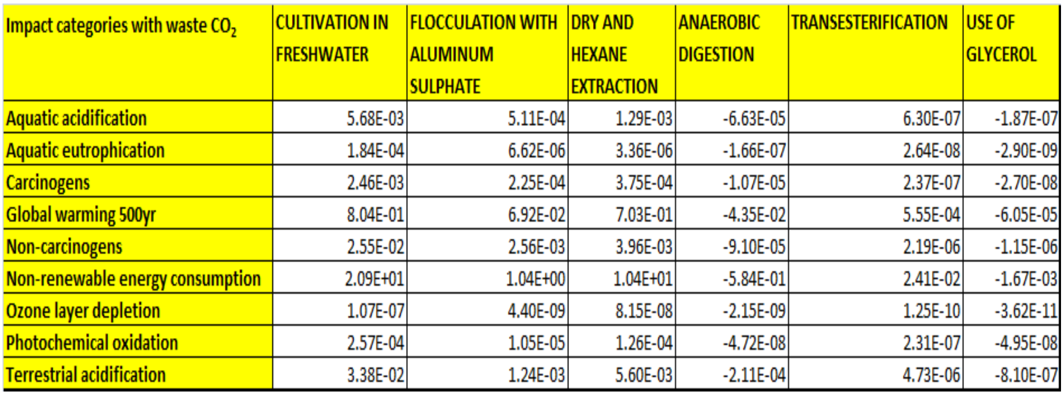
Figure 7.8: non renewable energy consumption for Case 2 when waste CO_2 is used. Comparison between algal biodiesel and diesel from fossil fuels (red column)

Table 7.8 and Table 7.9 show the total contributions of each phase to all impact categories in case that waste CO_2 is analyzed and “freshwater and wastewater scenarios” are alternatively considered. Excluding transesterification and glycerol use, all processes decrease their total contributions by about 50% on average when lipid content increases from 29% to 60%. The decrease of anaerobic digestion contributions means lower avoided impacts than Basic Case because the increase of the lipid content decreases the amount of the algal biomass produced.

In Table 7.8, GWP and aquatic acidification differ from Basic Case by about one order of magnitude (Table 5.5) whereas non renewable energy consumption, ODP, POCP and terrestrial AP show a difference with Basic Case by about 50%.

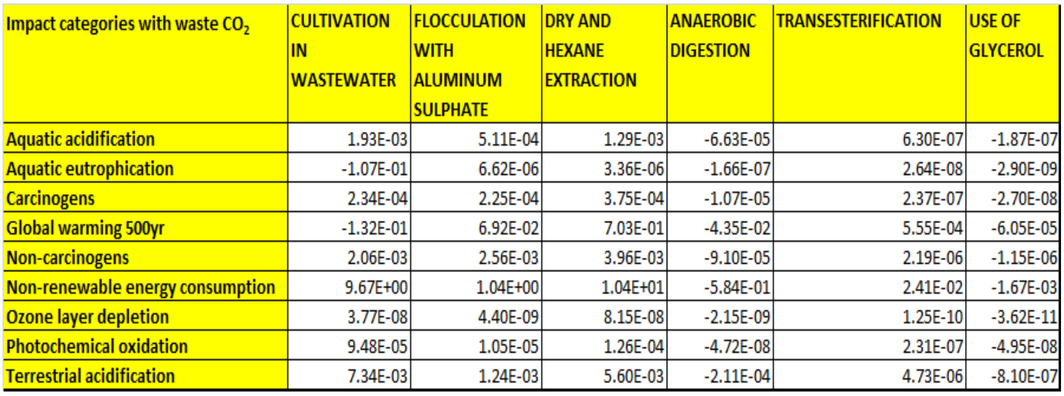
In comparison to the Basic Case, the use of waste CO_2 coupled with wastewater (Table 7.9) decreases the total contributions of cultivation to non renewable energy consumption, POCP and terrestrial AP by about one order of magnitude. On the other hand, the negative contribution of cultivation phase to GWP is decreased by about 50%.

Nevertheless, the relative weights of cultivation, aluminum flocculation anaerobic digestion and drying phase are equal to those of the Basic Case (see Figure 5.18 and Figure 5.20).



Impact categories with waste CO_2	CULTIVATION IN FRESHWATER	FLOCCULATION WITH ALUMINUM SULPHATE	DRY AND HEXANE EXTRACTION	ANAEROBIC DIGESTION	TRANSESTERIFICATION	USE OF GLYCEROL
Aquatic acidification	5.68E-03	5.11E-04	1.29E-03	-6.63E-05	6.30E-07	-1.87E-07
Aquatic eutrophication	1.84E-04	6.62E-06	3.36E-06	-1.66E-07	2.64E-08	-2.90E-09
Carcinogens	2.46E-03	2.25E-04	3.75E-04	-1.07E-05	2.37E-07	-2.70E-08
Global warming 500yr	8.04E-01	6.92E-02	7.03E-01	-4.35E-02	5.55E-04	-6.05E-05
Non-carcinogens	2.55E-02	2.56E-03	3.96E-03	-9.10E-05	2.19E-06	-1.15E-06
Non-renewable energy consumption	2.09E+01	1.04E+00	1.04E+01	-5.84E-01	2.41E-02	-1.67E-03
Ozone layer depletion	1.07E-07	4.40E-09	8.15E-08	-2.15E-09	1.25E-10	-3.62E-11
Photochemical oxidation	2.57E-04	1.05E-05	1.26E-04	-4.72E-08	2.31E-07	-4.95E-08
Terrestrial acidification	3.38E-02	1.24E-03	5.60E-03	-2.11E-04	4.73E-06	-8.10E-07

Table 7.8: Case 2: the total contribution of each process to all categories when waste CO_2 and freshwater are considered



Impact categories with waste CO_2	CULTIVATION IN WASTEWATER	FLOCCULATION WITH ALUMINUM SULPHATE	DRY AND HEXANE EXTRACTION	ANAEROBIC DIGESTION	TRANSESTERIFICATION	USE OF GLYCEROL
Aquatic acidification	1.93E-03	5.11E-04	1.29E-03	-6.63E-05	6.30E-07	-1.87E-07
Aquatic eutrophication	-1.07E-01	6.62E-06	3.36E-06	-1.66E-07	2.64E-08	-2.90E-09
Carcinogens	2.34E-04	2.25E-04	3.75E-04	-1.07E-05	2.37E-07	-2.70E-08
Global warming 500yr	-1.32E-01	6.92E-02	7.03E-01	-4.35E-02	5.55E-04	-6.05E-05
Non-carcinogens	2.06E-03	2.56E-03	3.96E-03	-9.10E-05	2.19E-06	-1.15E-06
Non-renewable energy consumption	9.67E+00	1.04E+00	1.04E+01	-5.84E-01	2.41E-02	-1.67E-03
Ozone layer depletion	3.77E-08	4.40E-09	8.15E-08	-2.15E-09	1.25E-10	-3.62E-11
Photochemical oxidation	9.48E-05	1.05E-05	1.26E-04	-4.72E-08	2.31E-07	-4.95E-08
Terrestrial acidification	7.34E-03	1.24E-03	5.60E-03	-2.11E-04	4.73E-06	-8.10E-07

Table 7.9: Case 2: the total contribution of each process to all categories when waste CO_2 and wastewater are considered

In conclusion:

1. Lipid content is an important parameter for biodiesel production. In fact**, the increase of lipid content decreases by about 50% both GHG emissions and energy consumption even if the environmental performance of algal biodiesel does not significantly improve.** Despite this improvement, the heat demand for drying phase is too high for making biodiesel production sustainable.
2. **When waste CO_2 and the potential use of wastewater are considered, the wet extraction highlights a GHG emissions savings**. The main limitation to develop these favorable scenarios is reaching a lipid content of 60% because it implies that algae cultivation should be stressed and low biomass productivity could be reached.
3. Increasing lipid content from 29% to 60%, **the total contributions of each phase decrease** to all impact categories in the scenarios analyzed.
4. **Total contributions of anaerobic digestion to all categories are lower** than those of the basic scenario, decreasing avoided impacts.
5. **The increase of lipid content and extraction efficiency** improve the environmental performance of biodiesel production but they **do not make algal biodiesel more environmentally sustainable than fossil fuels.**
6. The only interesting option is **"flocculation with aluminum sulphate and sCO_2 extraction" in case that waste CO_2 and wastewater are used**.

8 CONCLUSIONS

In this work, the environmental sustainability of the industrial production of algal biodiesel has been assessed and the processes needing improvement have been studied.

In particular, this work aims to assess the environmental sustainability of biodiesel production from microalgae cultivated in photobioreactors, locating the production in Denmark.

The main hindrance of this study is the lack of primary data. For this reason, secondary data were used and adapted in order to develop a biodiesel production system in Denmark.

The biodiesel sustainability has been assessed choosing the best available technologies and/or processes for algal biodiesel production. An evaluation of the parameters which most affect the biodiesel production has been performed.

In addition, a comparison between the environmental performances of fossil fuels and those of algal biodiesel has been carried out.

The results of this study aim to provide a realistic scenario of how such technology could be implemented in Denmark.

The system boundaries take into account the following processes: cultivation, harvesting, drying, oil extraction, anaerobic digestion, glycerol use and transesterification. In the cultivation phase, freshwater and wastewater are alternatively considered, creating different scenarios. Then for each scenario, the use of synthetic and waste CO_2 are alternatively assumed. In the harvesting phase, flocculation with aluminum sulphate or lime, and centrifugation are alternatively analyzed. In the oil extraction phase, hexane extraction or sCO_2 extraction have been considered. In this way, 24 scenarios are performed in this study.

The main relevant results are the following.

1. Obviously, **“wastewater scenarios” coupled with waste CO_2 have the lowest impacts** in all categories. The use of wastewater avoids the addition of nutrients to the water and the use of waste CO_2 avoids CO_2 production, which requires different inputs. **Aluminum flocculation and sCO_2 extraction coupled with the use of wastewater and waste CO_2 seem to have sustainable environmental performances.**
2. **Cultivation is the most impacting phase** in all “freshwater scenarios” coupled with synthetic CO_2. These impacts are due to the use of

nutrients added to water, synthetic CO_2 for algal growth and electricity demand. The environmental performances of cultivation improve when waste CO_2 is used. If wastewater and waste CO_2 are considered, cultivation contributions decrease significantly, reaching avoided impacts in GWP and EP. Only in this case, the drying phase and hexane extraction are the most relevant processes.

3. **Lime and aluminum flocculation appear interchangeable in terms of environmental impacts.**

Due to the aluminum sulphate toxicity, aluminum flocculation impacts mainly AP, carcinogens and non-carcinogens. Lime flocculation can be used as well but it requires a higher pH adjustment and it forms $CaCO_3$ precipitates, which have to be disposed of after harvesting.

In all scenarios, **centrifugation is the worst option for harvesting**, due to its large energy demand.

4. When the cultivation phase reduces its impact by the use of waste CO_2 and wastewater, the **drying phase and hexane extraction show relevant contributions to all impact categories**. These processes have the greatest impact on GWP, POCP, non-renewable energy consumption and ODP, because they require high amounts of thermal energy and electricity.

5. **sCO_2 extraction can be an interesting technology to develop** in this field. In all scenarios, sCO_2 extraction impacts less than hexane extraction. Since the wet extraction **does not require a drying phase**, sCO_2 extraction shows a better environmental performance than diesel in cases where wastewater and waste CO_2 are considered. As stated in section 5.3, the main limitation is the realization of high temperatures and high pressures, avoiding the formation of unsaturated compounds.

6. **Transesterification and glycerol use have negligible contributions** to all categories for all scenarios analyzed.

7. **Anaerobic digestion avoids impacts** in GWP, ODP, non-renewable energy consumption and AP. Obviously, wastewater and waste CO_2 improve its contributions, especially to GWP.

8. **The extraction efficiency is not a relevant parameter** for biodiesel production. Even if the environmental performance of biodiesel is better than that of the Basic Case, this difference is not significant because GWP and non-renewable energy consumption have differences within the same order of magnitude of the Basic Case.

9. **The increase of lipid content decreases GHG emissions and non-renewable energy consumption by about 50% but the environmental performances of algal biodiesel are still worse than diesel,** excluding the case in which aluminum flocculation is coupled with sCO_2 extraction (wastewater and waste CO_2). The main limitation to the development of these scenarios is reaching a lipid content of 60%, because it implies that algal cultivation should be stressed and low biomass productivity can be reached (Rodolfi et al., 2009).

In order to develop biodiesel production on an industrial scale, many improvements must be achieved. In particular, different aspects of cultivation need to be enhanced, such as the use of wastewater and waste CO_2 as a flue gas from an industrial power plant. Additionally, wet extraction is better than dry extraction since it requires a lower amount of energy. Cultivation in open ponds could be more attractive than PBA cultivation even if PBR shows higher algal biomass productivity. Hence, the use of PBR does not allow the achievement of environmental and energetic sustainability of algal biodiesel production. PBR can be used only for cultivation of algal inoculums or for other commercial products with higher market value.

REFERENCES

Aitken D., Antizar-Ladislao B., 2012. *Achieving a green solution: limitations and focus points for sustainable algal fuels.* Energies, vol. 5, pp. 1613-1647

Batan L., Quinn J., Willson B., Bradley T., 2010. *Net Energy and Greenhouse Gas Emission Evaluation of Biodiesel Derived from Microalgae*. Environmental Science & Technology, vol. 44, pp. 7975–7980

Borkowski MG., Zaimes GG., Khanna V., 2012. *Integrating LCA and Thermodynamic Analysis for Sustainability Assessment ofAlgal Biodiesel.* Sustainable Systems and Technology (ISSST). Proceedings of IEEE International Symposium. Boston, 16th-18th May 2012

Brentner LB., Eckelman MJ., Zimmerman JB., 2011. *Combinatorial Life Cycle Assessment to inform process design of industrial production of algal biodiesel.* Environmental Science & Technology, vol. 45, pp. 7060–7067

Brennan L., Owende P., 2013. *Biofuels from microalgae: towards meeting advanced fuel standards*. In Weifu Lee J., Advanced Biofuels and Bioproducts. Springer, New York, chapter 24, pp. 553-599

Chowdhury R., Viamajala S., Gerlach R., 2012. *Reduction of environmental and energy footprint of microalgal biodiesel production through material and energy integration*. Bioresource Technology, vol. 108, pp. 102-111

Clarens AF., Resurreccion EF., White MA., Colosi ML., 2010. *Environmental Life Cycle Comparison of Algae to Other Bioenergy Feedstocks.* Environmental Science & Technology, vol. 44, pp.1813–1819

Clarens A., Colosi L., 2013. *Life cycle assessment of algae-to-energy systems*. In Weifu Lee J., Advanced Biofuels and Bioproducts. Springer, New York, chapter 32, pp. 759-778

Collet P., Hélias A., Lardon L., Ras M., Goy RA., Steyer JP., 2011. *Life cycle assessment of microalgae culture coupled to biogas production.* Bioresource Technology, vol 102, pp. 207–214

Darzins, A., Philip P., Edye, L., 2010. *Current Status and Potential for Algal Biofuels Production. A report to IEA Bioenergy*. Task 39, pp. 1-144

De Morais MG., Costa JAV., 2007. *Isolation and selection of microalgae from coal fired thermoelectric power plant for biofixation of carbon dioxide.* Energy Conversion and Management, vol. 48, pp. 2169–2173

Demirbas A., 2010. *Use of algae as a biofuel sources.* Energy Conversion and Management, vol. 51, pp. 2738–2749

Douskova I., CDoucha J., Livansky K., Machat J., Novak P., Umysova D., Zachleder V., Vitova M., 2009. *Simulataneous flue gas bioremediation and reduction of microalgal biomass production costs*. Applied Microbiology and Biotechnology, vol. 82, pp. 179–185

Frank ED., Han J., Palou-Riviera I., Elgowainy A., 2012. *Methane and Nitrous oxide emissions affect the life cycle analysis of algal biofuels*. Environmental research letters, vol. 7, pp. 1-10

Ghasemi Y., Rasoul Amini S., Naseri AT., Montazeri-Najafabady N., Mobasher MA., Dabbagh F., 2012. *Microalgae Biofuel Potentials (Review)*. Applied Biochemistry and Microbiology, vol. 48, No. 2, pp. 126–144

Gonzalez-Fernandez C., Sialve B., Bernet N., Steyer JP., 2011. *Impact of microalgae characteristics on their conversion to biofuel. Part 1: focus on cultivation and biofuel production.* Biofuels, Bioproducts & Biorefining, vol. 6, pp. 105–113

Hannon M., Gimpel J., Tran M., Rasala B., Mayfield S., 2010. *Biofuels from algae: challenges and potential*. Biofuels, vol. 5, pp. 763-784

Herrero M., Cifuentes A., Ibanez E., 2006. S*ub- and supercritical fluid extraction of functional ingredients from different natural sources: plants, food-byproducts, algae and microalgae.* Food Chemistry, vol. 98, pp. 136-148

Holma A., Koponen K., Antikainen R., Lardon L., Leskinen P., Roux P., 2013. *Current limits of life cycle assessment framework in evaluating environmental sustainability - case of two evolving biofuel technologies*. Journal of Cleaner Production, vol. 54, pp. 215-228

ILCD, Handbook, International Reference Life Cycle Data System, 2010. *General guide for Life Cycle Assessment- detailed guidance.* European Commission, Joint Research Centre. Institute for Environment and Sustainability. European Union

Intergovernmental Panel on Climate Change, 2007. *An Assessment of the Intergovernmental Panel on Climate Change*, Synthesis Report.

Intergovernmental Panel on Climate Change’s fourth assessment report, 2007. *Changes in atmospheric constituents and radiative forcing,* chapter 2, pp. 133–134

ISO 14040, 2006. International Standard. *Environmental Management, Life Cycle Assessment, Principles and framework.* Geneva, Switzerland

ISO 14044, 2006. International Standard. *Environmental Management, Life Cycle Assessment, Requirements and Guidelines.* Geneva, Switzerland

Johnson DT., Taconi KA., 2007. *The glycerin glut: options for the value-added conversion of crude glycerol resulting from biodiesel production.* Environmental Progress, vol. 26, No. 4, pp. 338-348

Jørgensen A., Bikker P., Herrmann IT., 2012. *Assessing the greenhouse gas emissions from poultry fat biodiesel.* Journal of Cleaner Production, vol. 24, pp. 85-91

Jorquera O., Kiperstok A., Sales EA., Embiruçu M., Ghirardi ML., 2010. *Comparative energy life-cycle analyses og microalgal biomass production in open ponds and photobioreactors.* Bioresource Technology, vol. 101, pp. 1406–1413

Khoo HH., Sharratt PN., Das P., Balasubramanian RK., Naraharisetti PK., Shaik S., 2011. *Life cycle energy and CO_2 analysis of microalgae-to-biodiesel: Preliminary results and comparisons*. Bioresource Technology, vol. 102, pp. 5800–5807

Kröger M., Müller-Langer F., 2012. *Review on possible algal-biofuel production processes*. Biofuels, vol. 3, No. 3, pp. 333-349

Kumar A., Ergas S., Yuan X., Sahu A., Zhang Q., Dewulf J., Malcata FX., Van Langenhove H., 2010. *Enhanced CO_2 fixation and biofuel production via microalgae: recent developments and future directions*. Trends in Biotechnology, vol. 28, pp. 371–380

Lam MK., Lee KT., 2012. *Microalgae biofuels: A critical review of issues, problems and the way forward*. Biotechnology Advances, vol. 30, pp. 673–690

Langlois J., Sassi JF., Jard G., Steyer JP., Delgenes JP., Helias A., 2012. *Life cycle assessment of biomethane from offshorecultivated seaweed.* Biofuels, Bioproduct & Biorefining, vol. 6, pp. 387-404

Lardon L., Helias A., Sialve B., Steyer JP., Bernard O., 2009. *Life Cycle Assessment of Biodiesel Production from Microalgae.* Environmental Science & Technology, vol. 43, No. 17, pp.6475-6481

Life Cycle Assessment, An Operational Guide to the ISO Standards, 2001. Final report

Markevicius A., Katinas V., Perednis E., Tamasauskiene M., 2010. *Trends and sustainability criteria of the production and use of liquid biofuels*. Renewable & Sustainable Energy Reviews, vol. 14, pp. 3226‐3231

Mata TM., Martins AA., Sikdar SK., Costa CAV., Caetano NS., 2013. *Sustainability considerations about microalgae for biodiesel production*. .

In Weifu Lee J., Advanced Biofuels and Bioproducts. Springer, New York, chapter 31, pp. 745-757

Mendes RL., Fernandes HL., Coelho JP., Reis EC., Cabral JMS., Novais JM., Palavra AF., 1995. *Supercritical* CO_2 *extraction of carotenoids and other lipids from Chlorella vulgaris*. Food Chemistry, vol. 53, pp. 99–103

Menetrez MY., 2012. *Overview of Algae Biofuel Production and Potential Environmental Impact*. Environmental Science & Technology, vol. 46, pp. 7073-7085

Pagliaro M., Ciriminna R., Kimura H., Rossi M., Della Pina C., 2007. *From glycerol to value-added products.* Angewandte Chemie International Edition, vol. 46, pp. 4434-4440

Patil PD., Gude VG., Mannarswamy A., Cooke P., Nirmalakhandan N., Lammers P., Deng S., 2012. *Comparison of direct transesterification of algal biomass under supercritical methanol and microwave irradiation conditions.* Fuel, vol. 97, pp. 822–831

Pedroni PM., Beckert H., Benemann J., Bergman P., Davison J., 2001. *A proposal to establish an international network on biofixation of* CO_2 *and greenhouse gas abatement with microalgae*. Proceedings of the 1st national conference on carbon sequestration of the US Department of Energy-National Energy Technology. Washington (D.C.), 14th-17th May 2001

Pittman JK., Dean AP., Osundeko O., 2011. *The potential of sustainaable algal biofuel production using wastewater resources*. Bioresource Technology, vol. 102, pp. 17–25

Razon LF.,Tan RR., 2011. *Net energy analysis of the production of biodiesel and biogas from the microalgae: Haematococcus pluvialis and Nannochloropsis sp.*. Applied Energy, vol. 88, pp. 3507–3514

Rodolfi L., Chini Zittelli G., Bassi N., Padovani G., Biondi N., Bonini G., Tredici MR., 2009. *Microalgae for Oil: Strain Selection, Induction of Lipid Synthesis and Outdoor Mass Cultivation in a Low-Cost Photobioreactor.* Biotechnology and Bioengineering, vol. 102, No. 1, pp. 100-112

Sander K., Murthy GS., 2010. *Life cycle analysis of algal biodiesel*. The International Journal of Life Cycle Assessment, vol. 15, pp. 704–714

Santana A., Jesus S., Larrayoz MA., Filho RM., 2012. *Supercritical carbon dioxide extraction of algal lipids for the biodiesel production*. Procedia Engineering, vol. 42, pp. 1755-1761

Scott SA., Davey MP., Dennis JS., Horst I., Howe CJ., Smith DJL., Smith AG., 2010. *Biodiesel form algae: challenges and prospects.* Current Opinion in Biotechnology, vol. 21, pp. 277–286

Sevigné Itoiz E., Fuentes-Grunewald C., Gasol CM., Garces E., Alacid E., Rossi S., Rieradevall J., 2012. *Energy balance and environmental impact analysis of marine microalgal biomass production for biodiesel generation in a photobioreactor pilot plant*. Biomass & Bioenergy, vol. 39, pp. 324-335

Shirvani T., Yan X., Inderwildi OR., Edwards PP., King DA., 2011. *Life cycle energy and greenhouse gas analysis for algae-derived biodiesel*. Energy & Environmental Science, vol. 4, pp. 3773-3778

Sialve B., Bernet N., Bernard O., 2009. *Anaerobic digestion of microalgae as a necessary step to make microalgal biodiesel sustainable.* Biotechnology Advances, vol. 27, pp. 409–416

Singh A., Singh Nigam P., Murphy JD., 2010. *Mechanism and challenges in commercialisation of algal biofuels.* Bioresource Technology, vol. 102, pp. 26-34

Singh A., Singh Nigam P., Murphy JD.,2011. *Renewable fuels from algae: An answer to debatable land based fuels*. Bioresource Technology, vol. 102, pp. 10-16

Singh A., Pant D., Olsen SI., Singh Nigam P., 2012. *Key issues to consider in microalgae based biodiesel production*. Energy Education Science and Technology, Part A: Energy Science and Research, vol. 29, No. 1, pp. 687-700

Soratana K., Harper Jr. WF., Landis AE., 2012. *Microalgal biodiesel and the renewable fuel standard's greenhouse gas requirement*. Energy Policy, vol. 46, pp. 498-510

Stephens E., Ross IL., Hankamer B., 2013. *Expanding the microalgal industry – continuing controversy or compelling case?* Current Opinion in Chemical Biology, vol. 17, pp. 444-452

Stephenson AL., Kazamia E., Dennis JS., Howe CJ., Scott SA., Smith AG., 2010. *Life Cycle Assessment of Potential Algal Biofuel Production in the United Kingdom: a Comparison of Raceways and Air-Lift Tubular Bioreactors.* Energy Fuels, vol. 24, pp. 4062–4077

Sturm BSM. and Lamer SL., 2011. *An energy evaluation of coupling nutrient removal from wastewater with algal biomass production.* Applied Energy, vol. 88, pp. 3499-3506

Uduman N., Qi Y., Danquah MK., Forde GM., Hoadley A., 2013. *Dewatering of microalgal cultures: A major bottleneck to algae-based fuels*. Journal of Renewable and Sustainable Energy, vol. 2, pp. 1–15

Vasudevan V., Stratton RW., Pearlson MN., Jersey GR., Beyene AG., Weissman JC., Rubino M., Hileman JI., 2012. *Environmental Performance of Algal Biofuel Technology Options*. Environmental Science & Technology, vol. 46, pp. 2451-2459

Weingberg J., Kaltschmitt M., Wilhelm C., 2012. *Analysis of greenhouse gas emissions from microalgae-based biofuels*. Biomass Conversion and Biorefinery, vol. 2, pp. 179-194

Xu L., Brilman DWF., Withag JAM., Brem G., Kersten S., 2011. *Assessment of a dry and a wet route for the production of biofuels from microalgae: Energy balance analysis.* Bioresource Technology, vol. 102, pp. 5113–5122

Yang J., Xu M., Zhang X., Hu Q., Sommerfeld M., Chen Y., 2011. *Life-cycle analysis on biodiesel production from microalgae: Water footprint and nutrients balance.* Bioresource Technology, vol. 102, pp. 159–165

Zaimes GG., Khanna V., 2013. *Microalgal biomass production pathways: evaluation of life cycle environmental impacts*. Biotechnology for biofuels, vol. 6, pp. 1-11

9 APPENDIX

9.1 LCIA

Scenario 2, 3, 5, 6 are described in section 3.2.5.

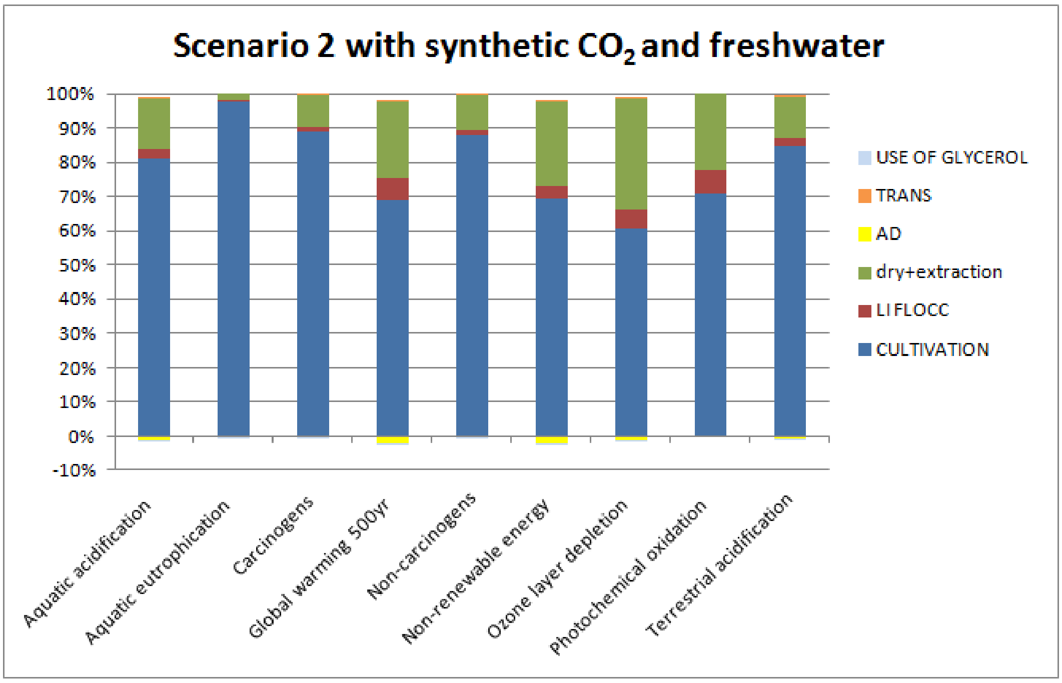


Figure 9.1: scenario 2. Contributions of each process to each impact category when synthetic CO_2 and freshwater are used. Li flocc describes flocculation with lime, dry+ extraction indicate drying phase and hexane extraction, trans is for transesterification and glycerol for the production of glycol propylene. AD indicates anaerobic digestion. Non renewable energy indicates non renewable energy consumption

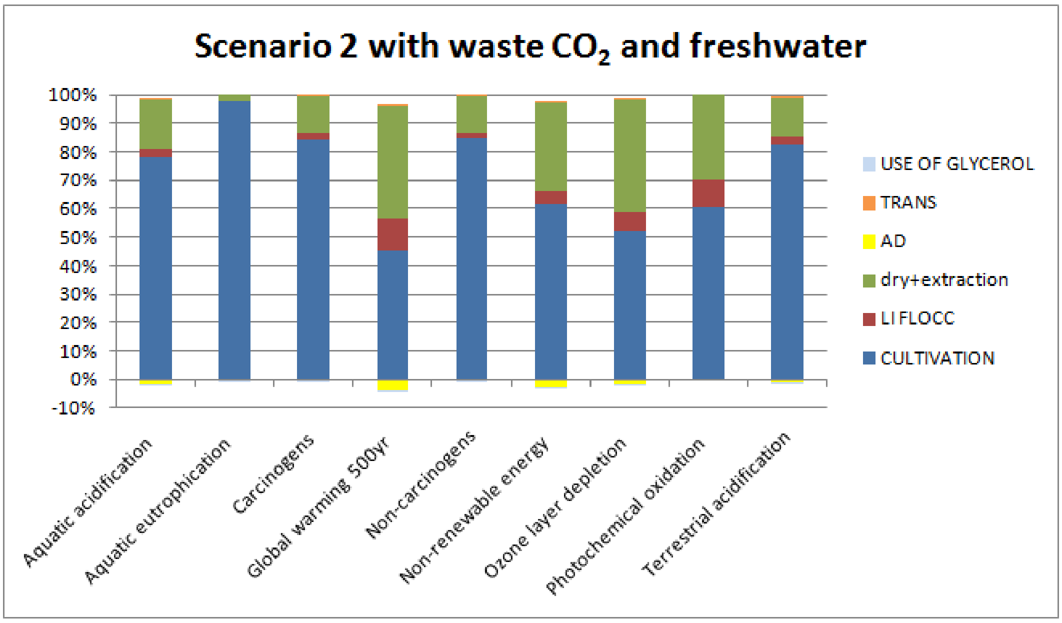


Figure 9.2: scenario 2. Contributions of each process to each impact category when waste CO_2 and freshwater are used. Li flocc describes flocculation with lime, dry+ extraction indicate drying phase and hexane extraction, trans is for

transesterification and glycerol for the production of glycol propylene. AD indicates anaerobic digestion. Non renewable energy indicates non renewable energy consumption

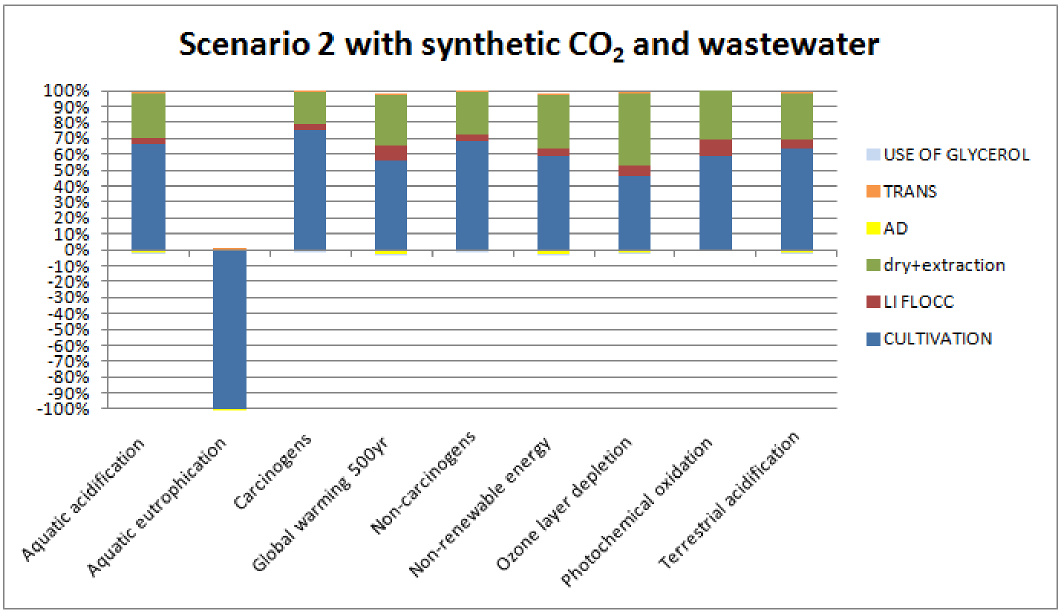


Figure 9.3: scenario 2. Contributions of each process to each impact category when synthetic CO_2 and wastewater are used. Li flocc describes flocculation with lime, dry+ extraction indicate drying phase and hexane extraction, trans is for transesterification and glycerol for the production of glycol propylene. AD indicates anaerobic digestion. Non renewable energy indicates non renewable energy consumption

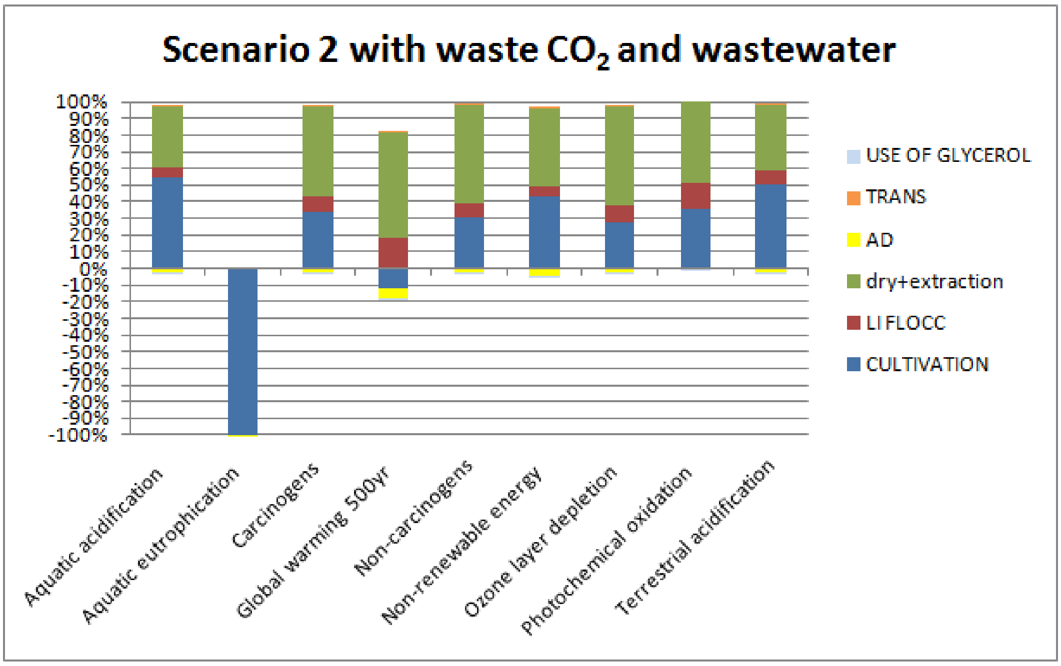


Figure 9.4: scenario 2. Contributions of each process to each impact category when waste CO_2 and wastewater are used. Li flocc describes flocculation with lime, dry+ extraction indicate drying phase and hexane extraction, trans is for transesterification and glycerol for the production of glycol propylene. AD indicates

anaerobic digestion. Non renewable energy indicates non renewable energy consumption

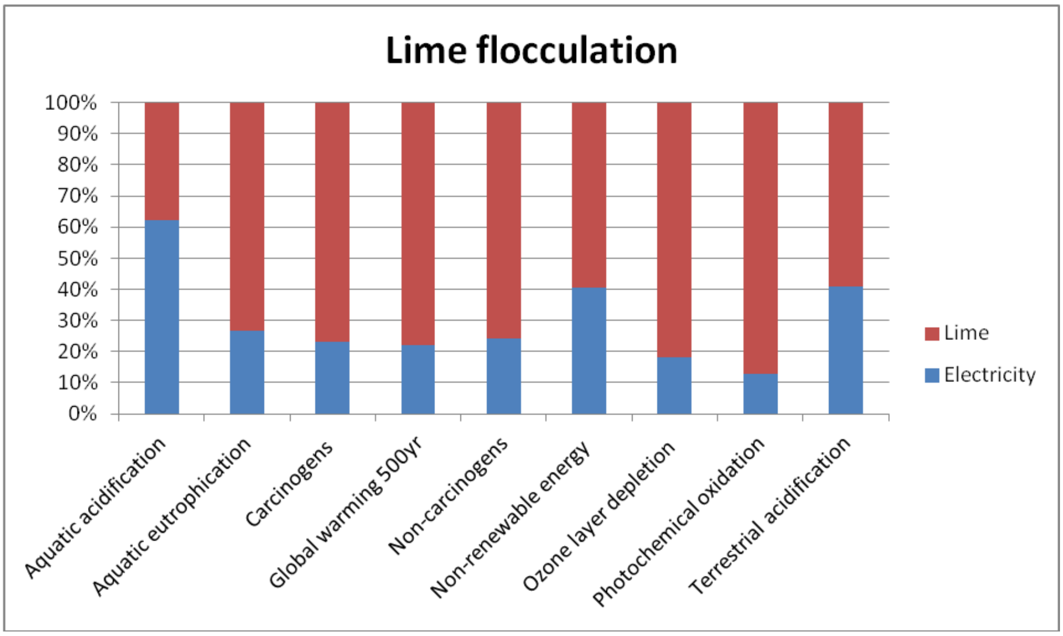


Figure 9.5: contribution of each process unit in lime flocculation

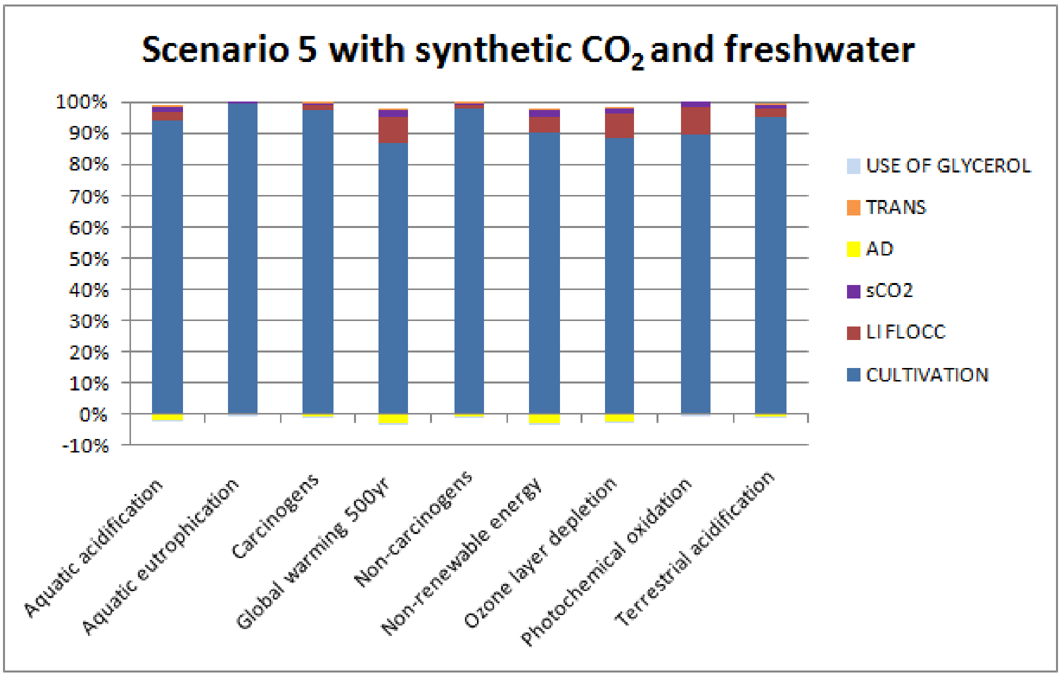


Figure 9.6: scenario 5. Contributions of each process to each impact category when synthetic CO_2 and freshwater are used. Li flocc describes flocculation with lime, sCO_2 indicates extraction with supercritical CO_2, trans is for transesterification and glycerol for the production of glycol propylene. AD indicates anaerobic digestion. Non renewable energy indicates non renewable energy consumption

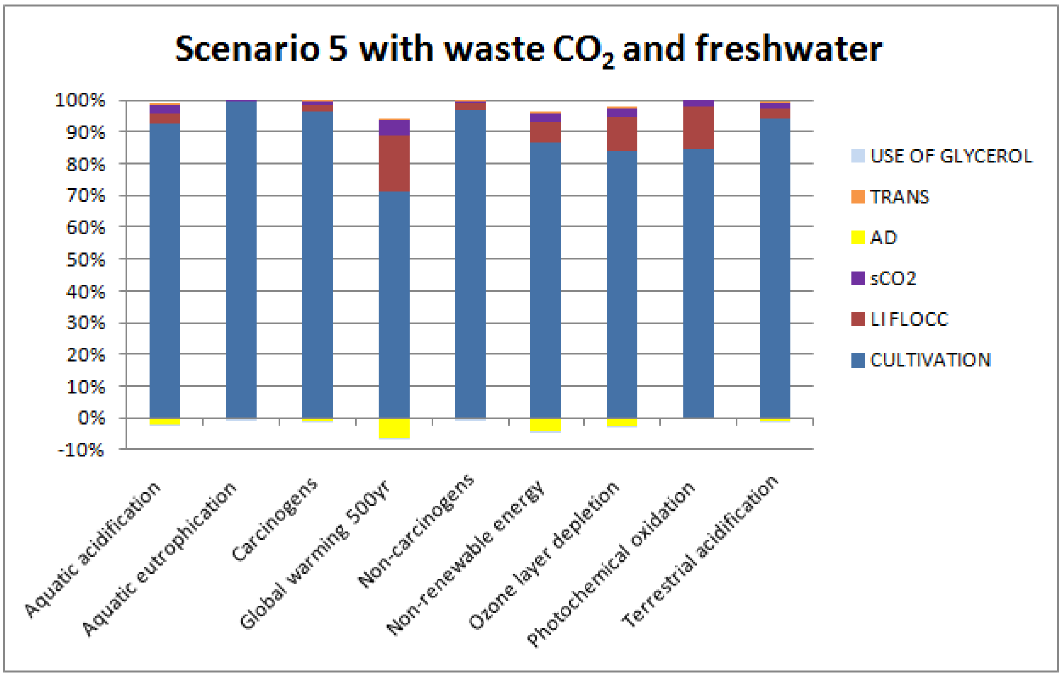


Figure 9.7: scenario. Contributions of each process to each impact category when waste CO_2 and freshwater are used. Li flocc describes flocculation with lime, sCO_2 indicates extraction with supercritical CO_2, trans is for transesterification and glycerol for the production of glycol propylene. AD indicates anaerobic digestion. Non renewable energy indicates non renewable energy consumption

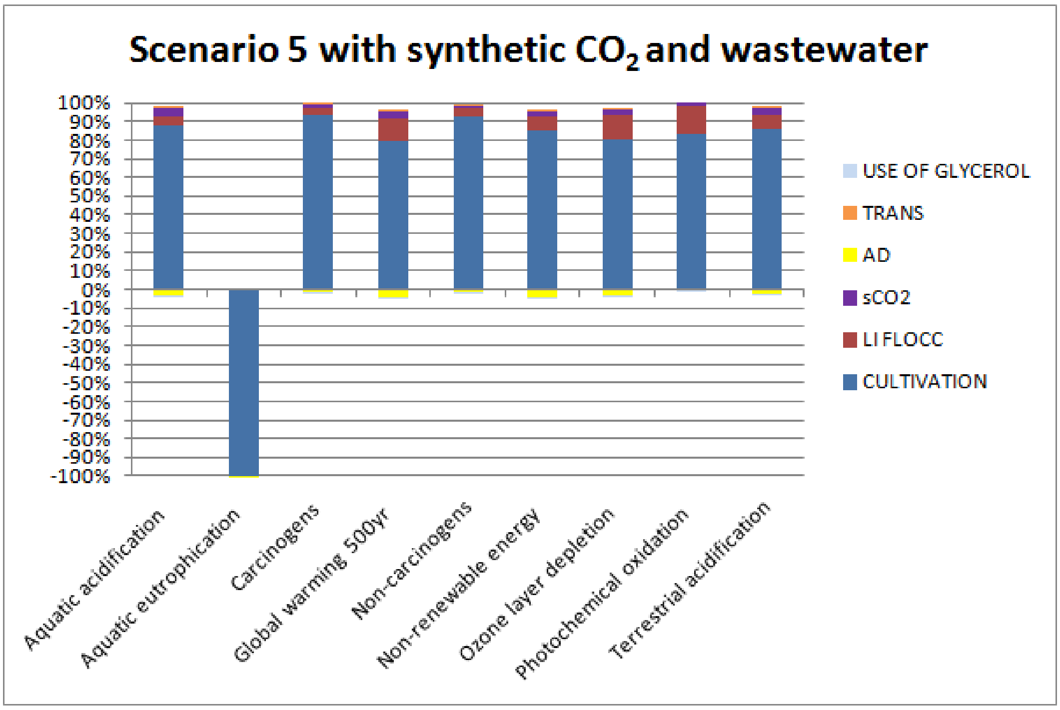


Figure 9.8: scenario 5. Contributions of each process to each impact category when synthetic CO_2 and wastewater are used. Li flocc describes flocculation with lime, sCO_2 indicates extraction with supercritical CO_2, trans is for transesterification and glycerol for the production of glycol propylene. AD indicates anaerobic digestion. Non renewable energy indicates non renewable energy consumption

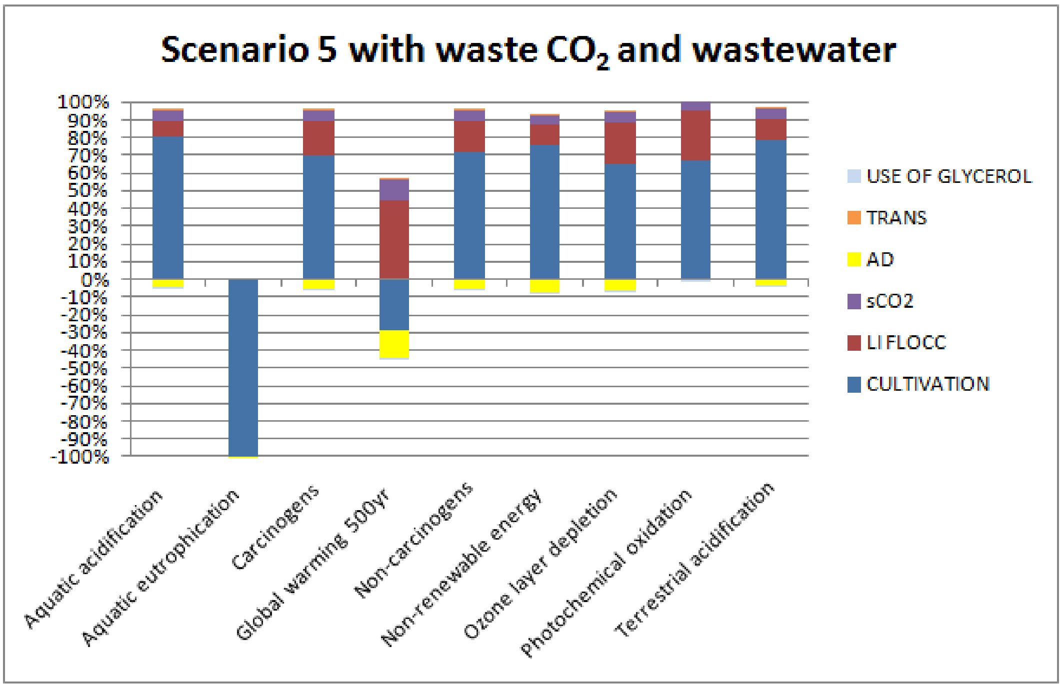


Figure 9.9: scenario 5. Contributions of each process to each impact category when waste CO_2 and wastewater are used. Li flocc describes flocculation with lime, sCO_2 indicates extraction with supercritical CO_2, trans is for transesterification and glycerol for the production of glycol propylene. AD indicates anaerobic digestion. Non renewable energy indicates non renewable energy consumption

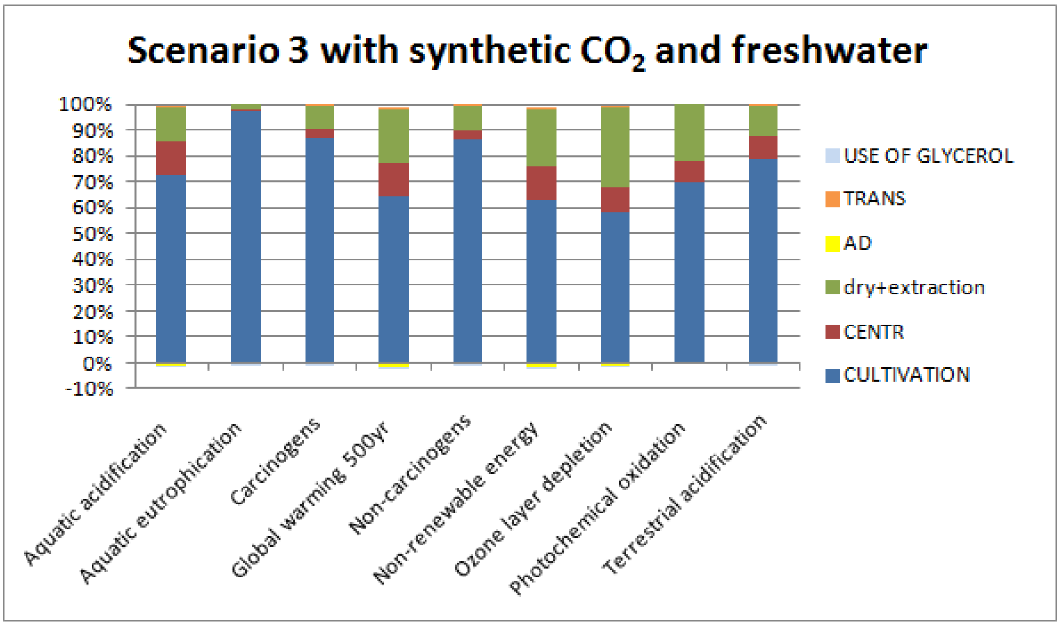


Figure 9.10: scenario 3. Contributions of each process to each impact category when synthetic CO_2 and freshwater are used. Centr means centrifugation. Dry+ extraction indicate drying phase and hexane extraction, trans is for transesterification and glycerol for the production of glycol propylene. AD indicates anaerobic digestion. Non renewable energy indicates non renewable energy consumption

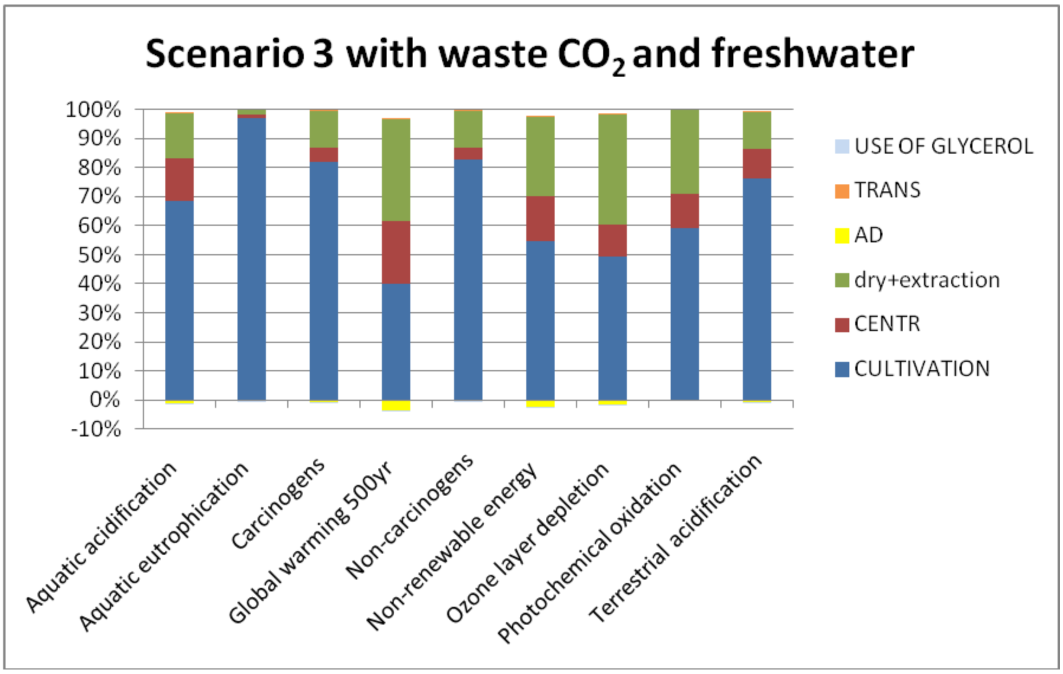


Figure 9.11: scenario 3. Contributions of each process to each impact category when waste CO_2 and freshwater are used. Centr means centrifugation. Dry+ extraction indicate drying phase and hexane extraction, trans is for transesterification and glycerol for the production of glycol propylene. AD indicates anaerobic digestion. Non renewable energy indicates non renewable energy consumption

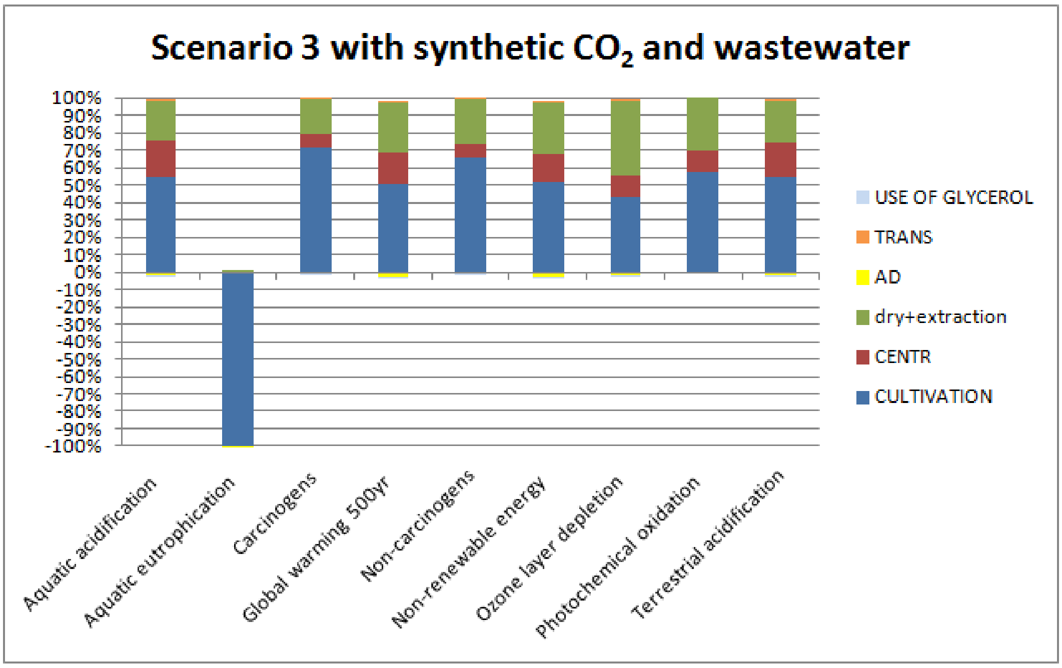


Figure 9.12: scenario 3. Contributions of each process to each impact category when synthetic CO_2 and wastewater are used. Centr means centrifugation. Dry+ extraction indicate drying phase and hexane extraction, trans is for transesterification and glycerol for the production of glycol propylene. AD indicates anaerobic digestion. Non renewable energy indicates non renewable energy consumption

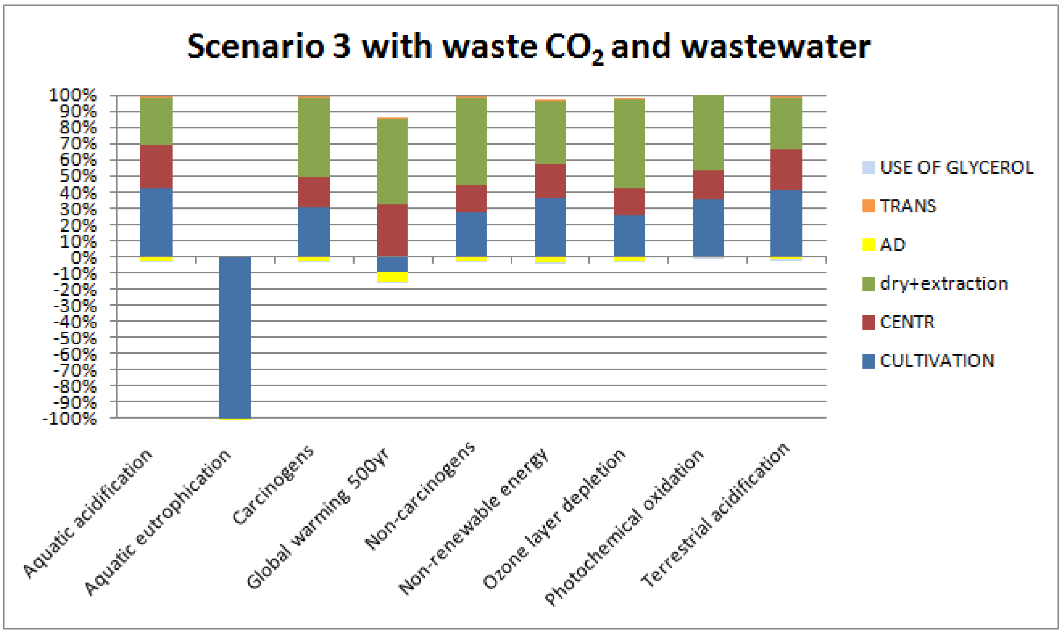


Figure 9.13: scenario 3. Contributions of each process to each impact category when waste CO_2 and wastewater are used. Centr means centrifugation. Dry+ extraction indicate drying phase and hexane extraction, trans is for transesterification and glycerol for the production of glycol propylene. AD indicates anaerobic digestion. Non renewable energy indicates non renewable energy consumption

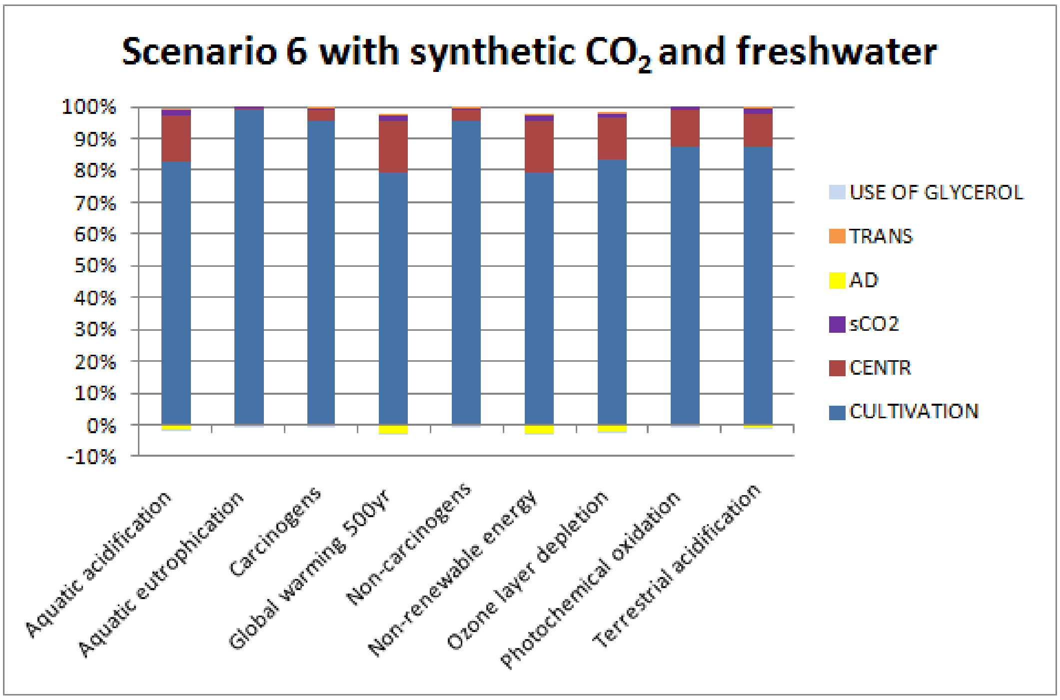


Figure 9.14: scenario 6. Contributions of each process to each impact category when synthetic CO_2 and freshwater are used. Centr means centrifugation. sCO_2 describes supercritical CO_2 extraction, trans is for transesterification and glycerol for the production of glycol propylene. AD indicates anaerobic digestion. Non renewable energy indicates non renewable energy consumption

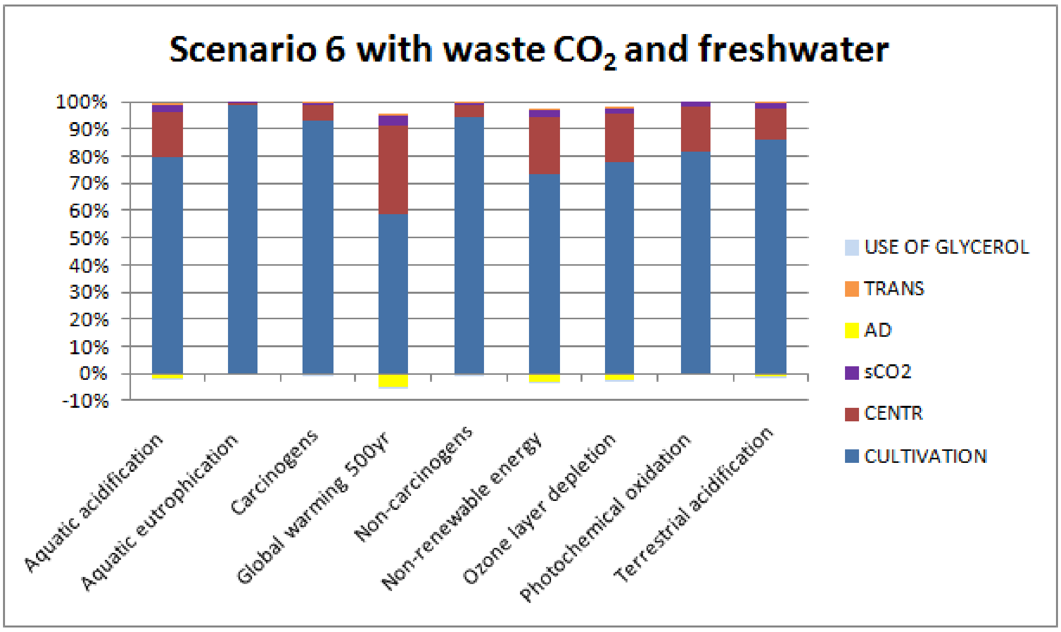


Figure 9.15: scenario 6. Contributions of each process to each impact category when waste CO_2 and freshwater are used. Centr means centrifugation. sCO_2 describes supercritical CO_2 extraction, trans is for transesterification and glycerol for the production of glycol propylene. AD indicates anaerobic digestion. Non renewable energy indicates non renewable energy consumption

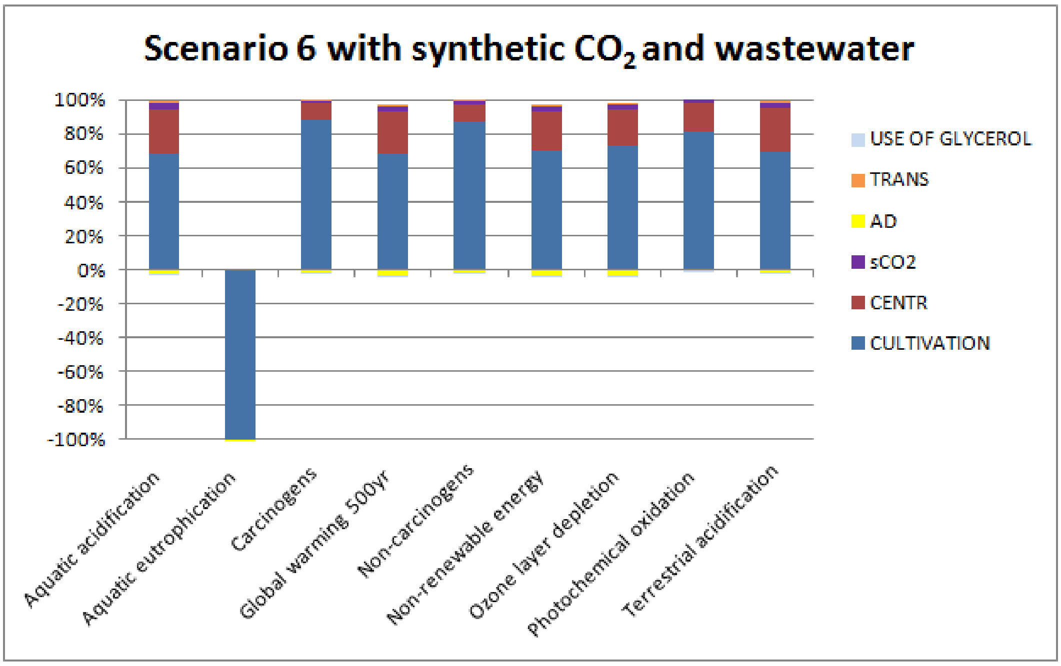


Figure 9.16: scenario 6. Contributions of each process to each impact category when synthetic CO_2 and wastewater are used. Centr means centrifugation. sCO_2 describes supercritical CO_2 extraction, trans is for transesterification and glycerol for the production of glycol propylene. AD indicates anaerobic digestion. Non renewable energy indicates non renewable energy consumption

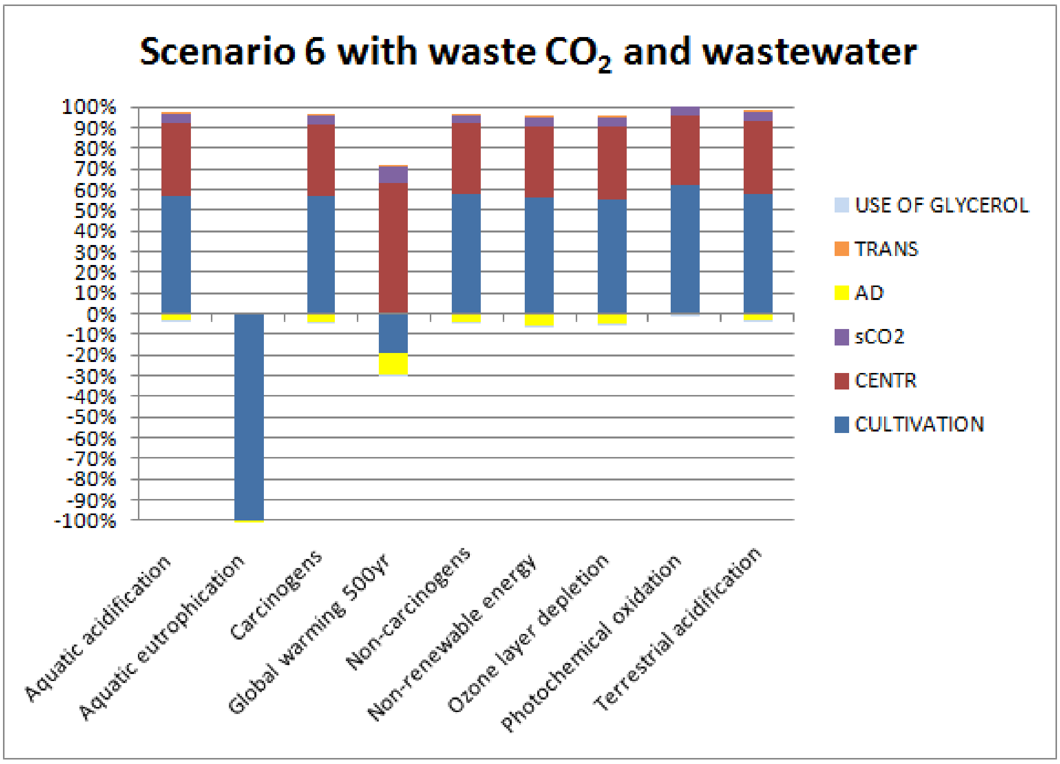


Figure 9.17: scenario 6. Contributions of each process to each impact category when waste CO_2 and wastewater are used. Centr means centrifugation. sCO_2 describes supercritical CO_2 extraction, trans is for transesterification and glycerol for the production of glycol propylene. AD indicates anaerobic digestion. Non renewable energy indicates non renewable energy consumption