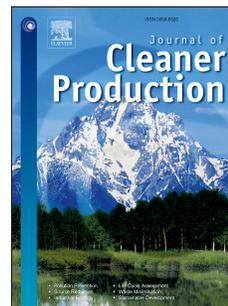


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Greenhouse gas emissions and energy balance of biodiesel production from microalgae cultivated in photobioreactors in Denmark: a life-cycle modeling

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4 *microalgae cultivated in photobioreactors in Denmark: a life-cycle modeling*

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20

21 **Abstract**

22 The current use of fossil fuels is problematic for both environmental and economic  
23 reasons and biofuels are regarded as a potential solution to current energy issues. This  
24 study analyzes the energy balances and greenhouse gas emissions of 24 different  
25 technology scenarios for the production of algal biodiesel from *Nannochloropsis*  
26 cultivated at industrial scale in photobioreactors in Denmark. Both consolidated and  
27 pioneering technologies are analyzed focusing on strengths and weaknesses which  
28 influence the performance. Based on literature data, energy balance and greenhouse  
29 gas emissions are determined in a comparative 'well-to-tank' Life Cycle Assessment  
30 against fossil diesel. Use of by-products from biodiesel production such as glycerol  
31 obtained from transesterification and anaerobic digestion of residual biomass are  
32 included. Different technologies and methods are considered in cultivation stage  
33 (freshwater vs. wastewater; synthetic CO<sub>2</sub> vs. waste CO<sub>2</sub>), harvesting stage  
34 (flocculation vs. centrifugation) and oil extraction stage (hexane extraction vs.  
35 supercritical CO<sub>2</sub> extraction). The choices affecting environmental performance of the  
36 scenarios are evaluated. Results show that algal biodiesel produced through current  
37 conventional technologies has higher energy demand and greenhouse gas emissions  
38 than fossil diesel. However, greenhouse gas emissions of algal biodiesel can be  
39 significantly reduced through the use of 'waste' flows (nutrients and CO<sub>2</sub>) but there are  
40 still technical difficulties with both microalgae cultivation in wastewater as well as  
41 transportation and injection of waste CO<sub>2</sub>. In any way, a positive energy balance is still  
42 far from being achieved. Considerable improvements must be made to develop an  
43 environmentally beneficial microalgae biodiesel production on an industrial scale. In  
44 particular, different aspects of cultivation need to be enhanced, such as the use of  
45 wastewater and CO<sub>2</sub>-rich flue gas from industrial power plants.

46

47 **HIGHLIGHTS**

- 48 • The best existing technologies for algal biodiesel production via PBRs have  
49 been compared.
- 50 • Fossil diesel has been taken as reference product.
- 51 • Energy balance and greenhouse gas emissions have been evaluated.
- 52 • Algal biodiesel has higher impacts compared to fossil diesel.
- 53 • Great improvements must be achieved to develop algal biodiesel on industrial  
54 scale

55 **KEYWORDS**

56 Biofuel; Renewable fuels; Biorefinery; Bioenergy; Biogas; *Nannochloropsis*

57

## 58 **1 Introduction**

59 The use of fossil fuels is increasingly problematic from both an economic and an  
60 environmental point of view. It has been necessary to identify compatible mitigation  
61 strategies to avoid the exhaustion of fossil fuels and minimize the excess of CO<sub>2</sub>  
62 emissions related to energy production (Ribeiro et al., 2007). In recent times, the  
63 European Commission has presented the EU Directive 2009/28/CE aiming to establish  
64 a target of 20% share of renewable energy sources in energy consumption by 2020. In  
65 this context at least 10% of the energy for transportation must be based on renewable  
66 energy sources (European Commission, 2009). As a renewable energy source,  
67 biofuels are an attractive alternative to current petroleum based fuels (Festel et al.,  
68 2014). Biofuels refer to liquid, gas and solid fuels derived from biomass, including a.o.  
69 dedicated energy crops, residues from agriculture, and algae. Biofuels are classified as  
70 first (from crop based feedstock), second (from non-food feedstock), third (from algae)  
71 and fourth (from genetically engineered crops) generation fuels on the basis of the  
72 biomass origin and production technology (Demirbas, 2011; Lü et al., 2011; Liew et al.,  
73 2014).

74 Due to several features, algae are regarded as a promising source of biofuels and are  
75 considered an interesting alternative to current biofuel crops (Singh et al., 2011; Aitken  
76 et al., 2014). The production of fuel from algae provides many advantages: algae do  
77 not compete with land use and crop production since they are aquatic organisms; their  
78 growth rate is higher than that of terrestrial plants from which the first-generation  
79 biofuels derive (Scott et al., 2010); they do not need chemicals, herbicides, pesticides  
80 for growth (Kumar et al., 2010; Yang and Chen, 2012); they can remove nitrogen and  
81 phosphorus from wastewater (Clarens et al., 2010); and, under certain conditions, such  
82 as nitrogen stress, algae are characterized by high lipid accumulation, a feature that  
83 increases biofuel production (Rodolfi et al., 2009).

84 On the other hand, there are several difficulties associated with the production of the  
85 third-generation biofuels and, until now, their commercial production has not been  
86 achieved on industrial scale in a cost-efficient manner (Biofuel.org.uk, 2010). Currently,  
87 only a few pilot plant projects have been developed (e.g. BFS Bio Fuel Systems, 2015;  
88 All-gas, 2012). At present, microalgae have been commercially cultivated only to obtain  
89 valuable products like carotenoids ( $\beta$ -carotene and astaxanthin) and long-chain poly-  
90 unsaturated fatty acids (Hannon et al., 2010). The main challenge that the algae biofuels  
91 sector is facing is to reduce capital and operating costs and so far only few studies  
92 have suggested the development of biodiesel production from microalgae on a  
93 commercial scale (Brentner et al., 2011; Seigné Itoiz et al., 2012).

94 Cultivation of microalgae can be done in open systems (lakes, ponds) or in controlled  
95 closed systems called photobioreactors (PBRs). Open ponds and lagoons have lower  
96 costs but also suffer from low productivity and contamination problems. PBRs enhance  
97 productivity, avoid cultivation contamination and are more reliable but they have high  
98 capital construction and operating costs (Demirbas, 2010; Benson et al., 2014). In both  
99 open and closed systems, there is a high energy requirement for mixing water with  
100 nutrients and CO<sub>2</sub> during the cultivation stage (Rodolfi et al., 2009). Moreover,  
101 harvesting and dewatering of biomass lead to high costs for production facilities as well  
102 as a high energy use (Brennan and Owende, 2013).

103 As part of the increasing research activities on algal biofuels, several Life Cycle  
104 Assessment (LCA) studies on biodiesel production from algae have been performed in  
105 order to assess their environmental performances. The results of these LCAs are  
106 conflicting, showing that only under specific conditions and assumptions the third-  
107 generation biofuels could be energetically and environmentally sustainable (Lardon et  
108 al., 2009; Khoo et al., 2011; Holma et al., 2013).

109 This study takes origin in the encouraging results obtained by Brentner et al., 2011 on  
110 flat panel PBRs hypothetically located in Phoenix, AZ. The location of PBRs has been

111 moved to Denmark, and a variety of technologies and implementation strategies to  
112 produce biodiesel from microalgae has been analyzed. Some of these technologies  
113 have already been developed on an industrial scale to produce valuable algal  
114 compounds while others are still on an experimental laboratory scale. Combining through  
115 different technologies in the different production stages, a total of 24 scenarios have  
116 been created. The energy demand and GHG emissions of the 24 scenarios and of the  
117 fossil diesel have been benchmarked and compared using a 'well-to-tank' life cycle  
118 approach. The sensitivity of some parameters that could affect biodiesel production  
119 have been evaluated.

120

## 121 **2 Material and methods**

122 This study applies Life Cycle Assessment (LCA) to evaluate the environmental  
123 performance of the different scenarios. LCA quantifies the environmental impacts of a  
124 product system considering its entire life cycle and is standardized by ISO  
125 14040/14044 (ISO, 2006a and 2006b). The method has four phases: goal and scope  
126 definition, life cycle inventory (LCI), life cycle impact assessment (LCIA), and  
127 interpretation of results. Below, data used and approaches applied in each of these  
128 phases are described.

### 129 *2.1 Goal and scope definition*

130 The goal of this LCA study was to assess algal biodiesel production on a hypothetical  
131 commercial scale by analyzing and comparing both consolidated (from algae-based  
132 industry) and pioneering technologies, focusing on strengths and weaknesses which  
133 influence the performance.

134 Assuming 39.35 MJ/kg as high heating value (HHV) (Brentner et al., 2011), the  
135 functional unit was 1 MJ of biodiesel and the system boundaries were 'well-to-tank' (i.e.  
136 from cultivation to biodiesel storage). The stages included were (Fig. 1): cultivation,

137 harvesting, drying, oil extraction, transesterification, anaerobic digestion of residual  
138 biomass with subsequent biogas combustion to generate energy (Zhang et al., 2013),  
139 and use of the by-product glycerol for the synthesis of propylene glycol. Substitution by  
140 system expansion was considered for biogas production and glycerol use. Substitution  
141 of glycerol in the production of propylene glycol has been chosen since this use is  
142 claimed to be the most economically attractive within the chemical industry (Pagliaro  
143 and Rossi, 2010). The algae selected was *Nannochloropsis* cultivated in flat panel  
144 PBRs and the production was assumed to be located in Denmark. Manufacturing,  
145 facilities maintenance, and use of infrastructures were not taken into account, except  
146 for the materials used for PBRs. The PBRs manufacturing is included since Seigné-  
147 Itoiz et al. (2012) state that construction of PBRs contributes significantly to energy use  
148 and environmental impacts. On the other hand, Brentner et al. (2011), who included  
149 also construction materials in the assessment, find that those materials contribute less  
150 than 1% to the cumulative energy demand (CED). The biodiesel combustion is not  
151 included by system boundaries.

## 152 *2.2 Life cycle inventory (LCI)*

153 All main inventory data are shown in Table 1. As indicated most of the data were  
154 compiled from previous works and were adapted to a Danish scenario (Table 2). The  
155 databases used for obtaining the additional process data were Gabi Professional 2006  
156 (PE International, 2007) and Ecoinvent 2.2 (Ecoinvent Centre, 2007).

### 157 *2.2.1 Scenarios*

158 A summary of the cultivation system and technologies assumed for each of the 24  
159 scenarios are reported in Table 3. As shown, cultivation in either freshwater (scenarios  
160 from 1 to 6) or wastewater (scenarios from 7 to 12) were considered. The algae require  
161 an injection of CO<sub>2</sub> into the growth medium for optimal growth and each scenario  
162 alternatively assumed the use of either pure CO<sub>2</sub> (where the carbon dioxide is delivered

163 in tanks) or waste CO<sub>2</sub> (named wCO<sub>2</sub>, with flue gas pumped from a nearby cement  
164 production plant into the PBRs). In the harvesting stage, three techniques were  
165 assessed: flocculation with aluminum sulfate (scenarios 1, 4, 7, 10), flocculation with  
166 lime (scenarios 2, 5, 8, 11), and centrifugation (scenarios 3, 6, 9, 12). Finally, both  
167 hexane extraction (scenarios 1, 2, 3, 7, 8, 9) and sCO<sub>2</sub> (supercritical CO<sub>2</sub>) extraction  
168 (scenarios 4, 5, 6, 10, 11, 12) were assessed in the oil extraction stage.  
169 Consolidated technologies of the current market (i.e. flocculation, centrifugation,  
170 extraction with hexane, algal cultivation in freshwater and with pure CO<sub>2</sub>) have thus  
171 been compared with advanced technologies not implemented on large scale (i.e. use of  
172 wastewater and waste CO<sub>2</sub>, and extraction with sCO<sub>2</sub>). The next sections, describe  
173 each stage in details.

### 174 *2.3 Algal biomass cultivation and harvesting*

175 Inventory data for cultivation and harvesting are showed in Table 1 and parameters  
176 used for modeling the *Nannochloropsis* cultivation in PBRs are illustrated in Table 4.  
177 The wastewater scenarios did not involve synthetic nutrients since wastewater is  
178 supposed to contain an adequate amount of nutrients to serve as a suitable growth  
179 medium for microalgae (Pittman et al., 2011). The CO<sub>2</sub> taken up during algal growth  
180 was subtracted from the total amount of CO<sub>2</sub> emissions in both 'pure CO<sub>2</sub>' and 'waste  
181 CO<sub>2</sub>' scenarios, whereas the CO<sub>2</sub> emissions from the production process of pure CO<sub>2</sub>  
182 are accounted for.  
183 The water content of wet algal biomass after harvesting is assumed to be about 70%  
184 (Singh et al., 2012).

### 185 *2.4 Drying and algal oil extraction*

186 Inventory data for drying and algal oil extraction are showed in Table 1. Drying stage  
187 was only assumed to be a requirement for hexane oil extraction since sCO<sub>2</sub> extraction  
188 is carried out directly from wet biomass (Xu et al., 2011; Mendes et al., 1995).

189 We assumed the use of thermal dryers with an energy consumption around 3.3 MJ per  
190 kilogram of evaporated water (Xu et al., 2011).

191 A dry biomass content in *Nannochloropsis* of 29% lipid, 10% carbohydrates and 30%  
192 proteins is hypothesized (Rodolfi et al., 2009; Razon and Tan, 2011). According to  
193 Brentner et al. (2011), the extraction efficiency with hexane is assumed to be 0.91.

194 Supercritical CO<sub>2</sub> for algal lipids extraction has been applied in laboratory on a number  
195 of algal species: *Skeletonema costatum* and *Ochromonas danica* (Polak et al., 1989),  
196 *Chlorella vulgaris* (Mendes et al., 1995), *Botryococcus braunii*, *Dunaliella salina*,  
197 *Arthrospira maxima* (Mendes et al., 2003), *Haematococcus pluvialis* (Thana et al.,  
198 2008). Recently, experiments have also been started on *Nannochloropsis sp.* (Andrich  
199 et al., 2005; Douglas, 2011; Crampon et al., 2013) but little information is reported on  
200 extraction efficiency even if the authors analyze the effects of operating conditions on  
201 the kinetics of the supercritical fluid extraction (Andrich et al., 2005; Crampon et al.,  
202 2013; Baskette, 2015). In scenarios assuming extraction with supercritical CO<sub>2</sub>, 27.5  
203 MPa and 47.5 °C were chosen as operating conditions (Mendes et al., 1995) and the  
204 extraction efficiency is assumed to be equal to the one with hexane (0.91). Neither  
205 hexane nor CO<sub>2</sub> recycling were considered in the LCI analysis.

### 206 *2.5 Transesterification and use of glycerol*

207 The amount of electricity and heat used in transesterification stage are shown in Table  
208 1. The conversion efficiency was hypothesized 98% (Brentner et al., 2011) and the  
209 catalyst used was methanol. The avoided production of propylene oxide has been  
210 calculated on the basis of the stoichiometric ratio and the process yields of the involved  
211 reactions. Data for propylene oxide to propylene glycol were from Ecoinvent 2.2  
212 (Ecoinvent Centre, 2007), data for glycerol to propylene glycol were from Pagliaro et al.  
213 (2007); the yields were 95% and 73%, respectively.

## 214 2.6 Life cycle impact assessment (LCIA)

215 The LCIA method applied was IMPACT 2002+ which proposes a feasible  
216 implementation of a combined midpoint/damage approach (Humbert et al., 2012). The  
217 chosen impact categories have been: global warming potential (GWP) and non-  
218 renewable energy consumption. For each scenario, the performances of algal biodiesel  
219 were compared with those of fossil diesel (from Ecoinvent 2.2; Ecoinvent Centre,  
220 2007).

## 221 2.7 Sensitivity analysis

222 The sensitivity analysis estimates the influence of assumptions, i.e. changes in input  
223 parameters, on the model outcome (ISO, 2006a; ISO, 2006b). Among all the possible  
224 parameters to be considered for the sensitivity analysis, we have selected two. The first  
225 parameter is the extraction efficiency ranging from 0.91 in the Base case (extraction  
226 efficiency with hexane, Brentner et al., 2011) to 0.95 in the Case 1 (extraction efficiency  
227 with supercritical CO<sub>2</sub>, Brentner et al., 2011).

228 The second parameter considered is the lipid content in the algal biomass which can  
229 vary dramatically as a result of the nitrogen supply (Jorquera et al., 2010; Khoo et al.,  
230 2011; Razon and Tan, 2011). The considered range of lipid content varies from 29%  
231 (lipid content experimentally observed in standard conditions by Rodolfi et al., 2009) to  
232 60% (lipid content experimentally observed under nitrogen deprivation conditions by  
233 Rodolfi et al., 2009).

234

## 235 3 Results and discussion

236 The results generally show that 'pure' CO<sub>2</sub> (grey columns, Fig.2 and Fig.3) causes  
237 GHG emissions and energy consumption at least 25%-30% higher than waste CO<sub>2</sub>  
238 (white columns, Fig.2 and Fig.3). This agrees well with the results obtained by  
239 Borkowski et al. (2012) which demonstrated that the use of waste CO<sub>2</sub> for algae

240 cultivation in PBR from a nearby power plant decreased GHG emissions by about 50%  
241 compared to the use of 'pure' CO<sub>2</sub>.

242 In general, GWP of biodiesel scenarios is one order of magnitude higher than GWP of  
243 fossil diesels (black column, Fig.2). Only the last three scenarios (Sc10-wCO<sub>2</sub>, Sc11-  
244 wCO<sub>2</sub> and Sc12-wCO<sub>2</sub>) show GHG emissions similar to or lower than those of fossil  
245 diesel. The last three scenarios achieve the best performances also considering non-  
246 renewable energy consumption (Fig. 3), even if this is considerably higher compared to  
247 fossil diesel. This indicates that the coupling of the 'waste flows' for algal cultivation  
248 with the use of sCO<sub>2</sub> for algal oil extraction – that avoids the drying stage – could be an  
249 interesting production system. The best scenario is Sc10-wCO<sub>2</sub> (flocculation with  
250 aluminum sulphate) which shows a negative GWP indicating a GHG sequestration and  
251 the lowest energy consumption. The result is in accordance with the studies by Lardon  
252 et al. (2009) which observed that only wet extraction can save GHG emissions in algal  
253 biodiesel production and by Vasudevan et al. (2012) which calculated very low GHG  
254 emissions (0.053 kg of CO<sub>2</sub> eq/MJ) when wet extraction was applied. Also Xu et al.  
255 (2011) observed that wet extraction dramatically decreases energy consumption.

256 Interesting information is provided by the 'non-renewable energy investment in energy  
257 delivered' (NEIED) (Yang and Chen, 2012). NEIED is expressed as the ratio between  
258 the non-renewable energy used directly and indirectly in the production process and  
259 the energy content in the biofuel. In this study the NEIED is >1 in all 24 scenarios. In  
260 particular, in our simulations algal biodiesel production requires from 20 MJ (Sc10-  
261 wCO<sub>2</sub>) to 90 MJ (Sc3-CO<sub>2</sub>) for producing 1 MJ of biodiesel. These values are very high  
262 but comparable with results obtained by other authors. Jorquera et al. (2010) find a  
263 consumption of about 14 MJ/MJ for tubular PBRs including only cultivation stage and  
264 Seigné Itoiz et al. (2012) report a consumption of 901 MJ/kg of DW biomass for indoor  
265 PBRs. In fact, cultivation in PBRs has a large energy demand due to the CO<sub>2</sub> pumping  
266 and nutrients mixing (Weinberg et al., 2012; Borkowski et al., 2012; Khoo et al., 2011).

267 Below we evaluate the relative contributions to GWP and non-renewable energy  
268 consumption of each stage in the worst (Sc3-CO<sub>2</sub>) and the best scenarios (Sc10-  
269 wCO<sub>2</sub>). The stages analyzed are: 1) algae cultivation; 2) harvesting; 3) (drying and) oil  
270 extraction; 4) transesterification; 5) anaerobic digestion (of residual biomass) and 6)  
271 use of glycerol.

272 Figure 4 illustrates the relative contributions in the worst scenario. As shown, the  
273 cultivation stage has the highest contribution to GWP and non-renewable energy  
274 consumption (62% and 66%, respectively), followed by drying and oil extraction (23%  
275 and 24%, respectively) and harvesting through centrifugation (15% and 13%,  
276 respectively). Anaerobic digestion contributes by avoiding GHG emissions and non-  
277 renewable energy consumption (both about -2%) while transesterification and use of  
278 glycerol in the propylene oxide industry do not give a relevant contribution. These  
279 results completely agree with previous studies. Many authors observed that cultivation  
280 (Batan et al., 2010; Borkowski et al., 2012; Weinberg et al., 2012), drying (Razon and  
281 Tan, 2011; Xu et al., 2011) and lipid extraction (Khoo et al., 2011) were the most  
282 impacting stages for biodiesel production both in terms of GHG emissions and energy  
283 requirements.

284 Figure 5 illustrates the best scenario. As far as GWP concerns, cultivation (-40%) and  
285 anaerobic digestion (-25%) contribute by avoiding GHG emissions while the most  
286 impacting stages are harvesting (15%) and sCO<sub>2</sub> extraction (15%). Transesterification  
287 and the glycerol use are negligible. Regarding non-renewable energy consumption, the  
288 most significant process is algae cultivation (92%) while the other stages have a very  
289 low contribution. The negative contribution of cultivation on GHG emissions is due to  
290 the sequestration of CO<sub>2</sub> in the algal cells and to the use of wastewater which eliminate  
291 the need of fertilizer production. However, these improvements do not eliminate the  
292 need of electric power during the cultivation stage. As a final result, in the best-case  
293 scenario we have an increment of the cultivation stage contribution to the energy

294 consumption. This is due to the fact that the energy demand of the algal harvesting and  
295 lipid extraction stages decreases in comparison to the worst case.

296

297 The percentage contribution analysis has identified three stages as the bottlenecks of  
298 algal biodiesel production: cultivation, drying and oil extraction, and harvesting.

299 Regarding the cultivation stage, the contribution of the different processes to the  
300 environmental impact are detailed in the Supplementary Data, figures 2.1, 2.2, 2.3 and

301 2.4. Electricity is always a significant contributor and when 'pure' CO<sub>2</sub> and/or nutrients  
302 are required these contribute significantly as well. The contributions of nutrients, CO<sub>2</sub>,

303 and electricity vary for the different scenarios. Contributions from construction  
304 materials, low density polyethylene (LDPE) sheets and reinforcing steel, are always

305 negligible. Considering the performances of Sc10-wCO<sub>2</sub>, Sc11-wCO<sub>2</sub> and Sc12-wCO<sub>2</sub>  
306 scenarios, it is evident that the capability to cultivate algae using waste flows (aqueous

307 and gaseous) plays a fundamental role for an environmental beneficial development of  
308 large scale biodiesel production from microalgae. Anyway, these technologies need to

309 be improved further to become efficient, affordable and accessible. Currently, the  
310 cultivation of algae in wastewater has not been developed on commercial scale yet but

311 only on pilot plants. Several challenges exists, e.g. the high turbidity of wastewater  
312 restricting the light penetration and making the algal cultivation inefficient (Pedroni et

313 al., 2001). Therefore, a water clarification pre-treatment could be necessary in order to  
314 reduce the presence of suspended matter and organic load (Pedroni et al., 2001). Also

315 the use of waste CO<sub>2</sub> is still experimental on a pilot scale. The main issues to be solved  
316 are the transfer of waste flue gas from an industrial plant to PBRs and the CO<sub>2</sub> losses

317 during this transfer. In fact, the energy demand for pumping the flue gas and the  
318 distance from the plant to PBRs limit this transfer (Pedroni et al., 2001). Moreover, it is

319 challenging to control the O<sub>2</sub>-concentration and the temperature which has to be  
320 reduced from above 100°C to app. 25°C (Dorminey, 2013). Additionally, flue gases

321 contain pollutants such as NO<sub>x</sub> and SO<sub>2</sub> which may have adverse effects on the algal  
322 species. However, first findings from studies reveal that the presence of pollutants in  
323 the flue gas in today's industrial emissions seems to be less of a problem in relation to  
324 the growth of the algae (Mortensen and Gislerød, 2014).

325

326 The drying and oil extraction stage is the second relevant bottleneck. Oil extraction with  
327 the sCO<sub>2</sub>-process decreases the impact contribution because it does not require drying  
328 of the algal biomass. Also in this case, the 'key' technology is very innovative and must  
329 be further enhanced. Mendes et al. (1995) observed that higher pressures and  
330 temperatures led to higher efficiencies in the extraction of lipids but Santana et al.  
331 (2012) found a correlation between the pressure and the presence of unsaturated  
332 compounds, i.e. high pressure leads to high amounts of unsaturated compounds in the  
333 algal oil thus reducing the biodiesel quality.

334

335 In terms of energy consumption and GHG emissions, the harvesting stage also played  
336 a significant role. In general, flocculation requires less energy than centrifugation; in  
337 particular, *Nannochloropsis* centrifugation has a large energy demand due to the small  
338 size of the cells (Rodolfi et al., 2009). This in line with Sander and Murthy (2010) who  
339 also identified a high energy demand of centrifugation (50% higher than flocculation) in  
340 comparison to other algal harvesting technologies such as separation or filtration.  
341 Flocculation with aluminum sulphate (scenarios 1, 4, 7, 10) and with lime (scenarios 2,  
342 5, 8, 11) show similar GHG emissions and energy performances, see Fig. 2 and Fig. 3.  
343 However, although flocculation requires less energy than centrifugation, both  
344 flocculants present some disadvantages. The main product of flocculation with  
345 aluminum sulphate is aluminum hydroxide which forms aggregates with algal biomass  
346 rendering it toxic for methanogens during anaerobic digestion (Demirbas, 2010). Even

347 if lime is less toxic than aluminum sulphate, it is less used for flocculation due to the  
348 precipitate formation, i.e.  $\text{CaCO}_3$ , in the water (Pedroni et al., 2001).

349 A possible improvement with respect to both flocculation and centrifugation could be  
350 the development of bio-flocculation (Pedroni et al., 2001). Bio-flocculation is biologically  
351 induced by bacteria (Lee et al., 2009). Recently, a naturally flocculating diatom  
352 *Skeletonema* was used to form flocs of *Nannochloropsis* (Schenk et al., 2008). Bio-  
353 flocculation is not toxic for microalgae, it requires low operating costs, and has a low  
354 energy demand. However, bio-flocculation is affected by environmental conditions  
355 which are the most relevant aspects to improve (Schenk et al., 2008).

#### 356 **4 Sensitivity analysis**

357 Tables 5A and 5B present the results of the sensitivity analysis. Increasing extraction  
358 efficiency from 0.91 to 0.95, results in lower values for GWP and non-renewable  
359 energy consumption (about 5% less than Basic Case).

360 Likewise, increasing the lipid content from 29% to 60% reduces both GHG emissions  
361 and energy consumption by app. 50%. Therefore, lipid content was confirmed as an  
362 important parameter for biodiesel production. Nonetheless, even with high lipid content  
363 the energy and GHG emissions performances of algal biodiesel are still inferior to  
364 those of diesel from fossil sources. These results are in agreement with the observation  
365 of Khoo et al. (2011) and Razon and Tan (2011). In particular, Khoo et al. (2011)  
366 demonstrated that increasing the lipid content by about 10% and 20% decreased the  
367 energy consumption by about 4% and 6%, respectively.

#### 368 **5 Conclusion**

369 Algal biodiesel produced through current conventional technologies shows higher  
370 energy demand and GHG emissions than those of fossil diesel. 'Wastewater scenarios'  
371 coupled with waste  $\text{CO}_2$  have the lowest impact in GHG-emissions and non-renewable  
372 energy consumption, in some cases even better than fossil diesel in terms of GHG-

373 emissions. However, a positive energy balance is still far from being achieved by algal  
374 biodiesel. Thus, further improvements are required in order to achieve a beneficial  
375 development of biodiesel production on an industrial scale. In particular, different  
376 aspects of cultivation need to be enhanced, such as the use of wastewater as source  
377 of nutrient and CO<sub>2</sub>-rich flue gas from industrial power plants as source of carbon. The  
378 research has been addressed towards algae cultivation with 'waste flows', that seems  
379 to be the key to reduce both the demand of energy and the GHG-emissions of  
380 biodiesel from microalgae. Additionally, the energy demand for mixing, pumping, etc. of  
381 the cultivation stage should be dramatically decreased. Considering the extraction,  
382 supercritical CO<sub>2</sub> extraction appears to be an interesting technology. However, further  
383 studies are needed to address the main limitations; how to achieve high temperatures  
384 and high pressures and still avoiding the formation of unsaturated compounds.

385

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569 **Table 1**

570 Summary of the inventory data for producing 1 MJ of algal biodiesel

571 (HHV=39.35 MJ/kg of biodiesel).

<b>FRESHWATER CULTIVATION</b>	<b>AMOUNT</b>	<b>UNIT</b>	<b>NOTES</b>
Carbon dioxide	0.61	kg	Calculated from 1
Tap water	0.47	m <sup>3</sup>	Calculated from 2
Electricity consumption	0.78	kWh	Calculated from 2
Ammonium nitrate	0.08	kg	Calculated from 3
Monocalcium phosphate	0.03	kg	Calculated from 3
<b>WASTEWATER CULTIVATION</b>			
Carbon dioxide	0.61	kg	Calculated from 1
Wastewater	0.47	m <sup>3</sup>	Calculated from 2
Electricity consumption	0.78	kWh	Calculated from 2
<b>FLOCCULATION</b>			
Electricity consumption	0.05	kWh	Calculated from 2
Aluminium sulphate	0.04	kg	Calculated from 4
Lime	0.15	kg	Calculated from 5
<b>CENTRIFUGATION</b>			
Electricity consumption	0.11	kWh	Calculated from 6
<b>DRYING</b>			
Heat	1.12	MJ	Calculated from 7
<b>EXTRACTION WITH HEXANE</b>			
Electricity consumption	0.01	kWh	Calculated from 2
Heat	0.10	MJ	Calculated from 2
Hexane	0.39	g	Calculated from 5
<b>SUPERCRITICAL CO<sub>2</sub> EXTRACTION</b>			
CO <sub>2</sub> liquid	3.7	g	Calculated from 8
Electricity consumption	0.18	kWh	Calculated from 9
<b>TRANSESTERIFICATION</b>			
Electricity consumption	0.001	kWh	Calculated from 2
Heat	0.02	MJ	Calculated from 2
Methanol	2.9	g	Calculated from 5

589 1: Wijffels and Barbosa, 2010

590 2: Brentner et al., 2011

591 3: Grobbelaar, 2004

592 4: Grima et al., 2003

593 5: Lardon et al., 2009

594 6: Foley et al., 2011

595 7: Xu et al., 2011

596 8: Mendes et al., 1995

597 9: Singh and Olsen, 2012

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599

600 **Table 2**

601 Parameters and processes used in the study adapted to the Danish situation.

PARAMETERS	AMOUNT	UNIT	REFERENCES
Denmark's electricity mix	-	-	Ecoinvent 2.2
Reference year of electricity mix	2004		Ecoinvent 2.2
Denmark's carbon intensity	0.2	kg CO <sub>2</sub> /2005 US \$	US EIA, 2015
Average solar irradiation in Denmark	3730	MJ/m <sup>2</sup> /y	Danish Meteorological Institute, 2013
Productivity days	200	n°	Danish Meteorological Institute, 2013
CO <sub>2</sub> emission from Danish cement industry	1420067	t/y	Singh and Olsen, 2012

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606 **Table 3**

607 Summary of cultivation systems and technologies used for each analysed  
 608 scenario.

CODE	CO <sub>2</sub> SOURCE	WATER SOURCE	HARVESTING MODE	EXTRACTION MODE
Sc1-CO <sub>2</sub>	Pure CO <sub>2</sub>	Tap water	Aluminum sulfate	With hexane
Sc1-wCO <sub>2</sub>	Waste CO <sub>2</sub>	Tap water	Aluminum sulfate	With hexane
Sc2-CO <sub>2</sub>	Pure CO <sub>2</sub>	Tap water	Lime	With hexane
Sc2-wCO <sub>2</sub>	Waste CO <sub>2</sub>	Tap water	Lime	With hexane
Sc3-CO <sub>2</sub>	Pure CO <sub>2</sub>	Tap water	Centrifugation	With hexane
Sc3-wCO <sub>2</sub>	Waste CO <sub>2</sub>	Tap water	Centrifugation	With hexane
Sc4-CO <sub>2</sub>	Pure CO <sub>2</sub>	Tap water	Aluminum sulfate	Supercritical CO <sub>2</sub>
Sc4-wCO <sub>2</sub>	Waste CO <sub>2</sub>	Tap water	Aluminum sulfate	Supercritical CO <sub>2</sub>
Sc5-CO <sub>2</sub>	Pure CO <sub>2</sub>	Tap water	Lime	Supercritical CO <sub>2</sub>
Sc5-wCO <sub>2</sub>	Waste CO <sub>2</sub>	Tap water	Lime	Supercritical CO <sub>2</sub>
Sc6-CO <sub>2</sub>	Pure CO <sub>2</sub>	Tap water	Centrifugation	Supercritical CO <sub>2</sub>
Sc6-wCO <sub>2</sub>	Waste CO <sub>2</sub>	Tap water	Centrifugation	Supercritical CO <sub>2</sub>
Sc7-CO <sub>2</sub>	Pure CO <sub>2</sub>	Wastewater	Aluminum sulfate	With hexane
Sc7-wCO <sub>2</sub>	Waste CO <sub>2</sub>	Wastewater	Aluminum sulfate	With hexane
Sc8-CO <sub>2</sub>	Pure CO <sub>2</sub>	Wastewater	Lime	With hexane
Sc8-wCO <sub>2</sub>	Waste CO <sub>2</sub>	Wastewater	Lime	With hexane
Sc9-CO <sub>2</sub>	Pure CO <sub>2</sub>	Wastewater	Centrifugation	With hexane
Sc9-wCO <sub>2</sub>	Waste CO <sub>2</sub>	Wastewater	Centrifugation	With hexane
Sc10-CO <sub>2</sub>	Pure CO <sub>2</sub>	Wastewater	Aluminum sulfate	Supercritical CO <sub>2</sub>
Sc10-wCO <sub>2</sub>	Waste CO <sub>2</sub>	Wastewater	Aluminum sulfate	Supercritical CO <sub>2</sub>
Sc11-CO <sub>2</sub>	Pure CO <sub>2</sub>	Wastewater	Lime	Supercritical CO <sub>2</sub>
Sc11-wCO <sub>2</sub>	Waste CO <sub>2</sub>	Wastewater	Lime	Supercritical CO <sub>2</sub>
Sc12-CO <sub>2</sub>	Pure CO <sub>2</sub>	Wastewater	Centrifugation	Supercritical CO <sub>2</sub>
Sc12-wCO <sub>2</sub>	Waste CO <sub>2</sub>	Wastewater	Centrifugation	Supercritical CO <sub>2</sub>

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611 **Table 4**612 Parameters used for modelling the *Nannochloropsis* cultivation in PBRs.

PARAMETERS	AMOUNT	UNIT	REFERENCES
<i>Nannochloropsis</i> productivity	0.27	kg/m <sup>3</sup> /day	Jorquera et al., 2010
Biomass productivity	37.8	t/ha/year	Singh and Olsen, 2012
Number of PBR	2667	per hectare	Brentner et al., 2011
PBR length	2.5	m	Brentner et al., 2011
PBR height	1.5	m	Brentner et al., 2011
PBR thick	0.070	m	Brentner et al., 2011
PBR volume	0.263	m <sup>3</sup>	Brentner et al., 2011
Residence time	2.6	days	Brentner et al., 2011
Area	3.75	m <sup>2</sup>	Brentner et al., 2011
LDPE sheet	0.011	kg/kg biomass	Brentner et al., 2011
Life time	50	years	Brentner et al., 2011
Steel	0.00085	kg/kg biomass	Brentner et al., 2011
Life time	50	years	Brentner et al., 2011

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614

615 **Table 5A**

616 Results of the sensitivity analysis for GWP (kg CO<sub>2</sub>-eq). Basic case (91%  
 617 extraction efficiency and 29% lipid content) compared to the increase of  
 618 extraction efficiency (95%) and lipid content (60%). The functional unit is 1 MJ  
 619 of biodiesel.

CODE	BASIC CASE	EXTRACTION EFFICIENCY 95%	LIPID CONTENT 60%
Sc1-CO <sub>2</sub>	5.95E+00	5.70E+00	2.90E+00
Sc1-wCO <sub>2</sub>	3.11E+00	2.98E+00	1.53E+00
Sc2-CO <sub>2</sub>	6.23E+00	5.97E+00	3.04E+00
Sc2-wCO <sub>2</sub>	3.39E+00	3.25E+00	1.67E+00
Sc3-CO <sub>2</sub>	6.71E+00	6.43E+00	3.28E+00
Sc3-wCO <sub>2</sub>	3.88E+00	3.72E+00	1.90E+00
Sc4-CO <sub>2</sub>	4.60E+00	4.41E+00	2.25E+00
Sc4-wCO <sub>2</sub>	1.77E+00	1.69E+00	8.83E-01
Sc5-CO <sub>2</sub>	4.88E+00	4.68E+00	2.39E+00
Sc5-wCO <sub>2</sub>	2.05E+00	1.96E+00	1.02E+00
Sc6-CO <sub>2</sub>	5.37E+00	5.14E+00	2.62E+00
Sc6-wCO <sub>2</sub>	2.54E+00	2.43E+00	1.25E+00
Sc7-CO <sub>2</sub>	4.01E+00	3.84E+00	1.97E+00
Sc7-wCO <sub>2</sub>	1.18E+00	1.13E+00	5.96E-01
Sc8-CO <sub>2</sub>	4.29E+00	4.11E+00	2.10E+00
Sc8-wCO <sub>2</sub>	1.46E+00	1.40E+00	7.32E-01
Sc9-CO <sub>2</sub>	4.78E+00	4.58E+00	2.34E+00
Sc9-wCO <sub>2</sub>	1.94E+00	1.86E+00	9.67E-01
Sc10-CO <sub>2</sub>	2.66E+00	2.55E+00	1.32E+00
Sc10-wCO <sub>2</sub>	-1.67E-01	-1.60E-01	-8.32E-02
Sc11-CO <sub>2</sub>	2.94E+00	2.82E+00	1.45E+00
Sc11-wCO <sub>2</sub>	1.13E-01	1.08E-01	5.61E-02
Sc12-CO <sub>2</sub>	3.43E+00	3.29E+00	1.69E+00
Sc12-wCO <sub>2</sub>	5.99E-01	5.74E-01	3.02E-01

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623 **Table 5B**

624 Results of the sensitivity analysis for non-renewable energy consumption (MJ).

625 Basic case (91% extraction efficiency and 29% lipid content) compared to the

626 increase of extraction efficiency (95%) and lipid content (60%). The functional

627 unit is 1 MJ of biodiesel.

CODE	BASIC CASE	EXTRACTION EFFICIENCY 95%	LIPID CONTENT 60%
Sc1-CO <sub>2</sub>	8.27E+01	7.92E+01	4.03E+01
Sc1-wCO <sub>2</sub>	6.51E+01	6.24E+01	3.18E+01
Sc2-CO <sub>2</sub>	8.36E+01	8.01E+01	4.08E+01
Sc2-wCO <sub>2</sub>	6.60E+01	6.33E+01	3.23E+01
Sc3-CO <sub>2</sub>	9.27E+01	8.88E+01	4.52E+01
Sc3-wCO <sub>2</sub>	7.51E+01	7.20E+01	3.67E+01
Sc4-CO <sub>2</sub>	6.26E+01	6.00E+01	3.06E+01
Sc4-wCO <sub>2</sub>	4.50E+01	4.32E+01	2.21E+01
Sc5-CO <sub>2</sub>	6.36E+01	6.09E+01	3.11E+01
Sc5-wCO <sub>2</sub>	4.60E+01	4.41E+01	2.26E+01
Sc6-CO <sub>2</sub>	7.26E+01	6.96E+01	3.55E+01
Sc6-wCO <sub>2</sub>	5.51E+01	5.28E+01	2.70E+01
Sc7-CO <sub>2</sub>	5.93E+01	5.69E+01	2.91E+01
Sc7-wCO <sub>2</sub>	4.18E+01	4.00E+01	2.06E+01
Sc8-CO <sub>2</sub>	6.03E+01	5.78E+01	2.95E+01
Sc8-wCO <sub>2</sub>	4.27E+01	4.09E+01	2.10E+01
Sc9-CO <sub>2</sub>	6.94E+01	6.65E+01	3.39E+01
Sc9-wCO <sub>2</sub>	5.18E+01	4.96E+01	2.54E+01
Sc10-CO <sub>2</sub>	3.93E+01	3.77E+01	1.94E+01
Sc10-wCO <sub>2</sub>	2.17E+01	2.08E+01	1.09E+01
Sc11-CO <sub>2</sub>	4.02E+01	3.86E+01	1.98E+01
Sc11-wCO <sub>2</sub>	2.26E+01	2.17E+01	1.13E+01
Sc12-CO <sub>2</sub>	4.83E+01	4.73E+01	2.42E+01
Sc12-wCO <sub>2</sub>	3.17E+01	3.04E+01	1.57E+01

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631 **Figure captions**

632 Fig. 1 System boundaries of biodiesel production and the most important flows  
633 used for each stage.

634 Fig. 2 GWP (kg CO<sub>2</sub>-eq) of all 24 scenarios. CO<sub>2</sub> indicates the use of 'pure' CO<sub>2</sub>  
635 (grey column) for algae cultivation whereas wCO<sub>2</sub> specifies the use of waste  
636 CO<sub>2</sub> (white column) in microalgae cultivation stage. All scenarios have been  
637 compared to fossil diesel (black column, Ecoinvent Centre, 2007).

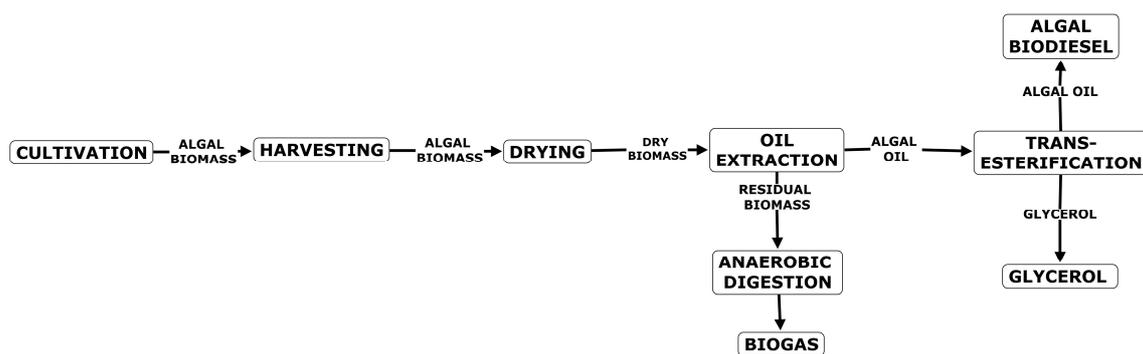
638 Fig. 3 Non-renewable energy consumption (MJ) of all 24 scenarios. CO<sub>2</sub>  
639 indicates the use of industrial CO<sub>2</sub> (grey column) for algae cultivation whereas  
640 wCO<sub>2</sub> specifies the use of waste CO<sub>2</sub> (white column) in microalgae cultivation  
641 stage. All scenarios have been compared to fossil diesel (black column,  
642 Ecoinvent Centre, 2007).

643 Fig. 4 Relative contribution of each stage of the worst scenario, which assumed  
644 the use of freshwater and 'pure' CO<sub>2</sub> for algae cultivation, centrifugation for  
645 algal harvesting and algal oil extraction with hexane. (Read the legend from top  
646 to bottom)

647 Fig. 5 Relative contributions of each stage of the best scenario, which assumed  
648 the use of wastewater and waste CO<sub>2</sub> for algae cultivation, flocculation with  
649 aluminium sulphate for algal harvesting and sCO<sub>2</sub> extraction in algal oil  
650 extraction. (Read the legend from top to bottom)

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654 Fig. 1

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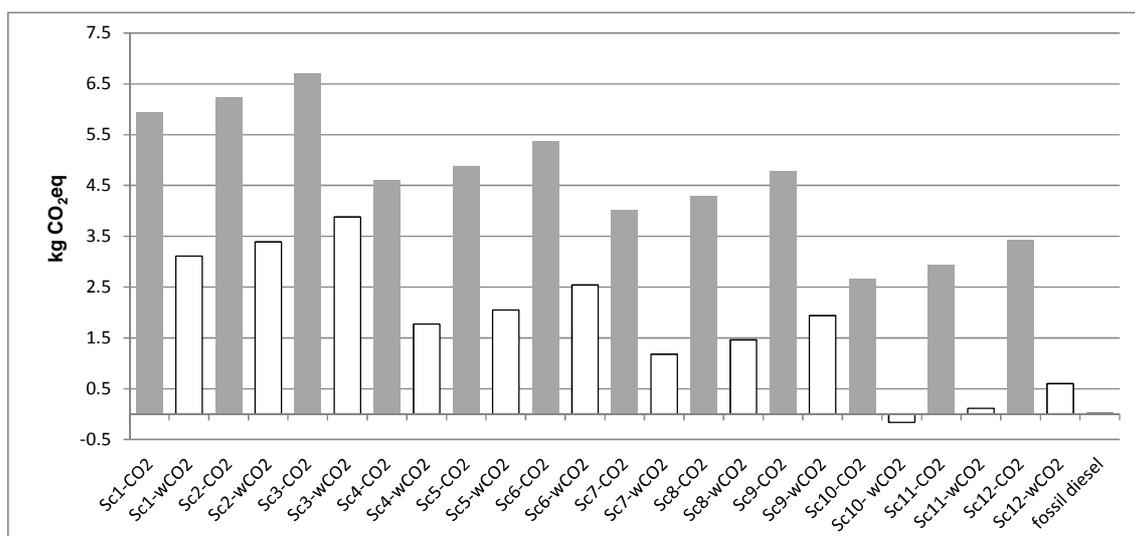
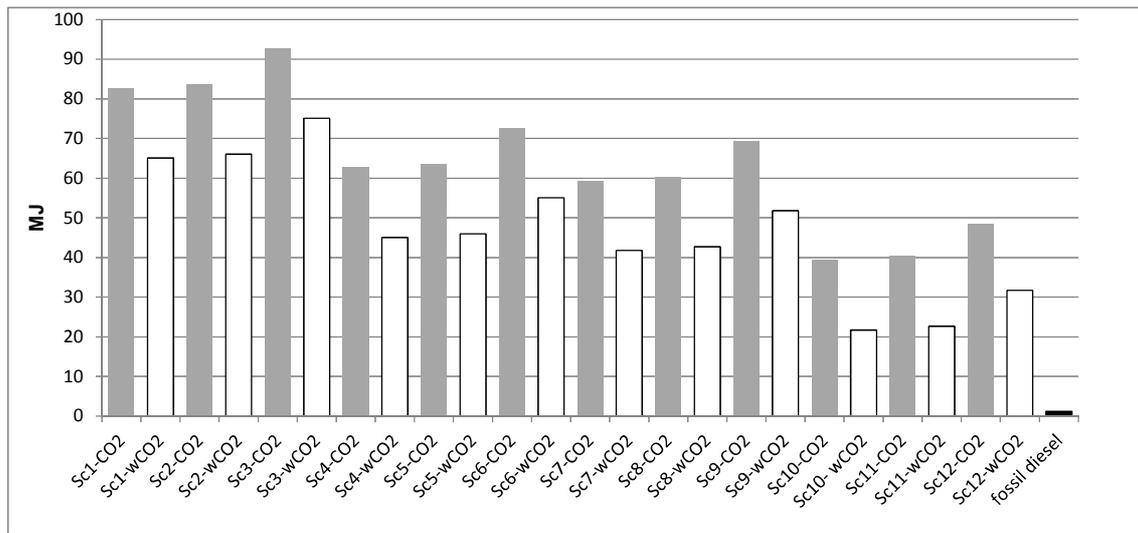
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Fig. 2

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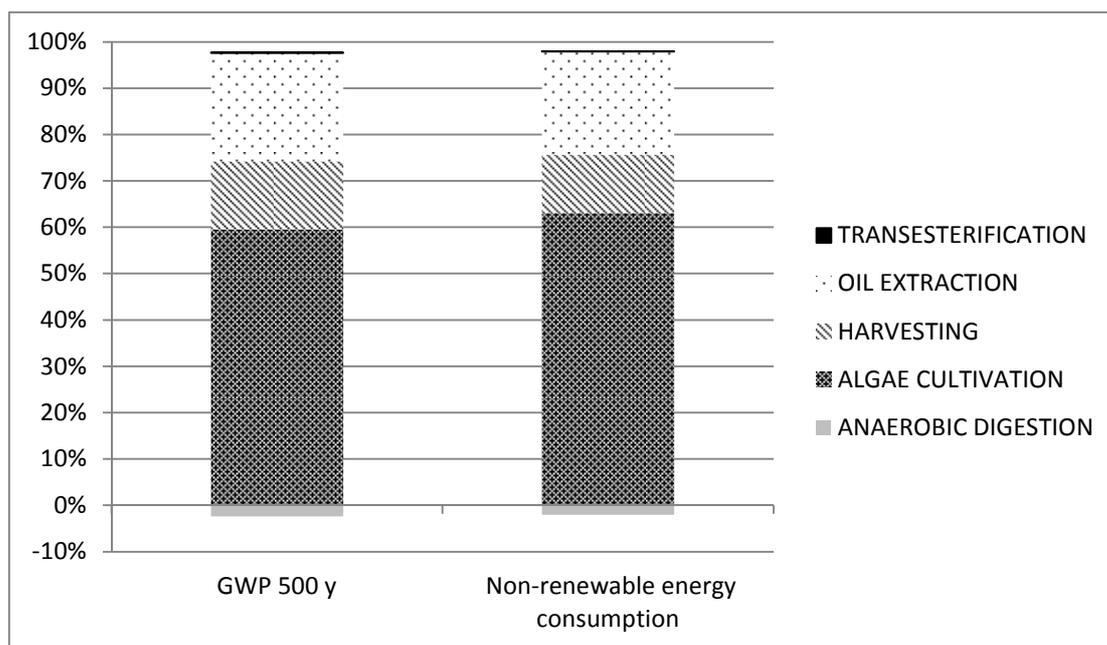


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662 Fig. 3

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Fig. 4

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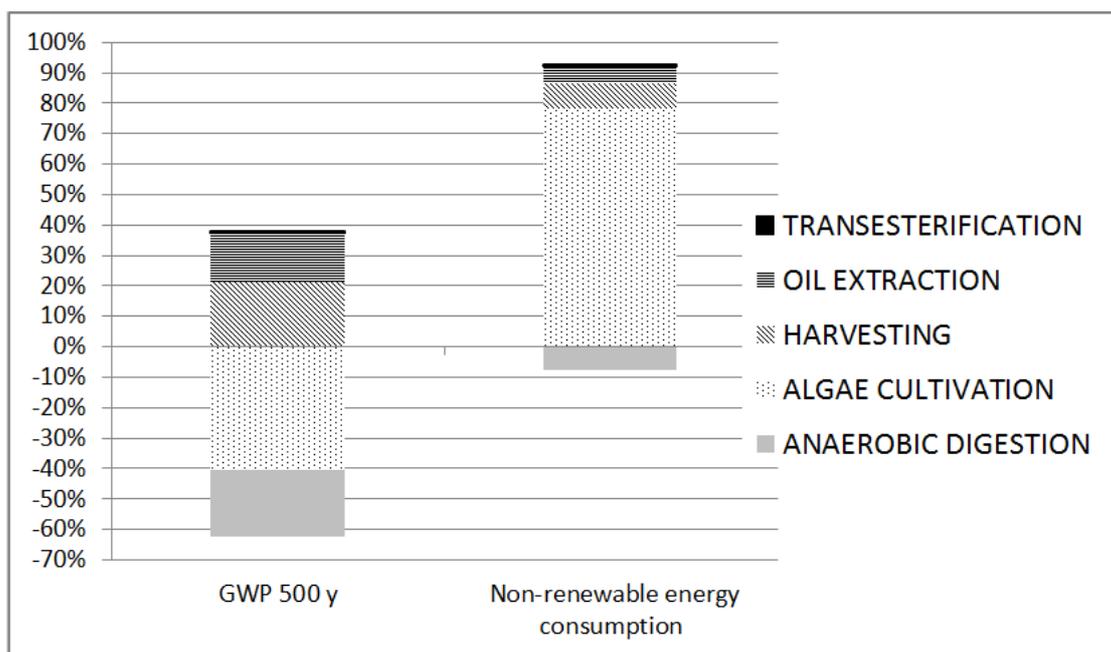
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Fig. 5

Supporting information for “*Application of LCA approach to Energy and Greenhouse Gas Emission impact of biodiesel production from microalgae cultivated in PBRs: a case study in Denmark*” submitted by Monari et al. (2013)

### 1. Detailed description of LCI data

The following detailed tables describe which flows are used and their correspondent processes in Gabi and which database has been used. The processes considered are cultivation (Table 1.1), harvesting and drying (Table 1.2), algal oil extraction (Table 1.3), transesterification (Table 1.4), anaerobic digestion (Table 1.5) and glycerol use (Table 1.6).

<b>FLOWS USED FOR CULTIVATIO</b>		
<b>Flows</b>	<b>Process in Gabi</b>	<b>Database</b>
Carbon dioxide (CO <sub>2</sub> )	RER: carbon dioxide liquid at plant	Ecoinvent
Water	RER: tap water at user	Ecoinvent
Total electricity consumption in cultivation	DK: electricity production mix	Ecoinvent
LDPE sheet	RER: polyethylene LDPE, granulate at plant	Ecoinvent
Steel	RER: reinforcing steel at plant	Ecoinvent
Ammonium nitrate	RER: ammonium nitrate, as N, at regional storehouse	Ecoinvent
Mono calcium phosphate	RER: single superphosphate, as P <sub>2</sub> O <sub>5</sub> , at regional storehouse	Ecoinvent
<b>WASTEWATER CULTIVATION</b>		
Water	Water (wastewater, untreated) [Production residues in life cycle]	Ecoinvent
Nitrogen	Nitrogen (N-compounds) [Inorganic emissions to air]	Ecoinvent
Phosphorus	Phosphorus [Inorganic emissions to air]	Ecoinvent

**Table 1.1: cultivation phase**

<b>HARVESTING</b>		
<b>Flows used for harvesting</b>	<b>Process in Gabi</b>	<b>Database</b>
Electricity consumption in flocculation	DK: Electricity production mix	Ecoinvent
Aluminium sulphate	RER: aluminium sulphate powder at plant	Ecoinvent
Lime	CH: lime hydrated packed at plant	Ecoinvent
<b>CENTRIFUGATION</b>		
Electricity consumption in centrifugation	DK: Electricity production mix	Ecoinvent
<b>DRYING</b>		
Heat	RER: heat, unspecific at chemical plant	Ecoinvent

Table 1.2: harvesting and drying phases

<b>EXTRACTION WITH HEXANE</b>		
<b>Flows for algal oil extraction</b>	<b>Process in Gabi</b>	<b>Database</b>
Electricity consumption in hexane extraction	DK: electricity production mix	Ecoinvent
Heat	RER: heat unspecific at plant	Ecoinvent
Hexane	RER: hexane at plant	Ecoinvent
<b>SCO<sub>2</sub> EXTRACTION</b>		
CO <sub>2</sub> liquid	RER: carbon dioxide liquid at plant	Ecoinvent
Electricity	DK: electricity production mix	Ecoinvent

Table 1.3: algal oil extraction phase

<b>TRANSESTERIFICATION</b>		
<b>Flow</b>	<b>Process in Gabi</b>	<b>Database</b>
Electricity consumption	DK: Electricity production mix	Ecoinvent
Heat	RER: Heat unspecific at plant	Ecoinvent
Methanol	GLO: methanol at plant	Ecoinvent

Table 1.4: transesterification phase

<b>ANAEROBIC DIGESTION</b>		
<b>PRODUCTION OF BIOGAS</b>		
<b>Flow</b>	<b>Process in Gabi</b>	<b>Database</b>
Electricity	CH: electricity, low voltage, at grid	Ecoinvent
Plant for Anaerobic digestion	CH: anaerobic digestion plant, biowaste	Ecoinvent
Transport	CH: transport, lorry 20-28t, fleet average	Ecoinvent
Transport for municipal waste	CH: transport, municipal waste collection, lorry 21t	Ecoinvent
Heat	RER: heat, natural gas, at boiler condensing modulating >100kW	Ecoinvent
Municipal solid waste	CH: disposal, municipal solid waste, 0 % water, to municipal incineration [municipal incineration]	Ecoinvent
Biogas from biowaste	CH: biogas, from biowaste, at storage [fuels]	Ecoinvent
<b>ELECTRICITY FROM BIOGAS</b>		
Lubricating oil	RER: lubricating oil, at plant	Ecoinvent
Cogen unit for electricity	RER: cogen unit 160kWe, components for electricity only	Ecoinvent
Disposal of oil	CH: disposal, used mineral oil, 10% water, to hazardous waste incineration	Ecoinvent
Cogen unit for electricity and heat	RER: cogen unit 160kWe, common components for heat+electricity	Ecoinvent
Biogas	CH: biogas, production mix, at storage [fuels]	Ecoinvent

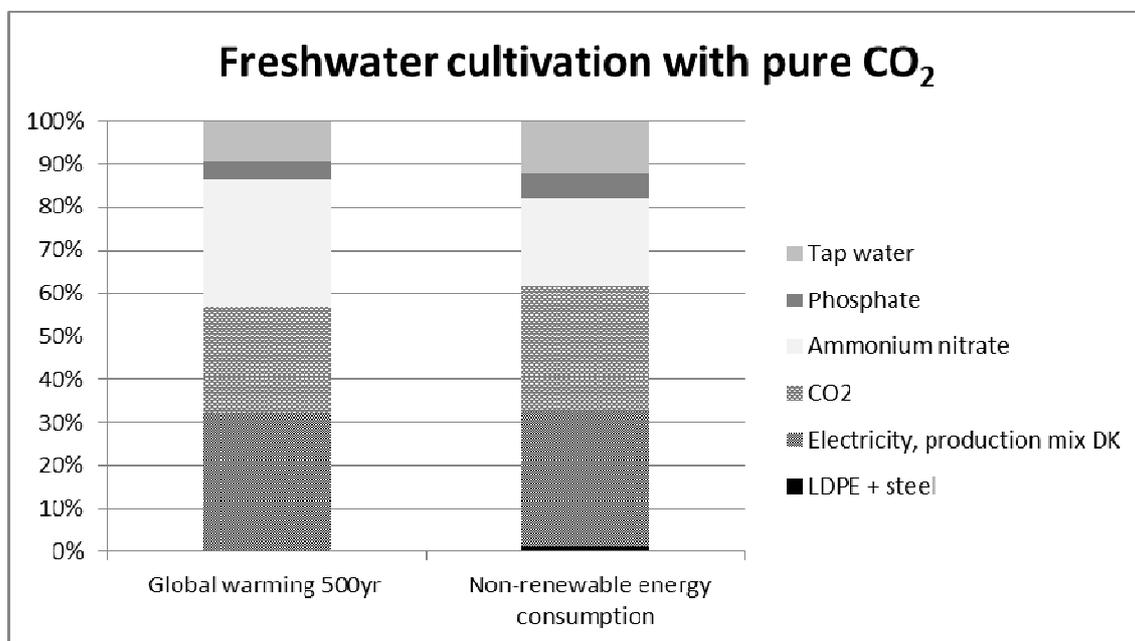
Table 1.5: anaerobic digestion

USE OF GLYCERINE TO PRODUCE PROPYLENE GLYCOL		
Flow	Process in Gabi	Database
Electricity use	UCTE: electricity, medium voltage, production UCTE, at grid [production mix]	Ecoinvent
Heat	RER: heat, natural gas, at industrial furnace >100kW	Ecoinvent
Transport in street	RER: transport, lorry >16t, fleet average [Street]	Ecoinvent
Transport in railway	RER: transport, freight, rail [Railway]	Ecoinvent
Chemical plant	RER: chemical plant, organics	Ecoinvent

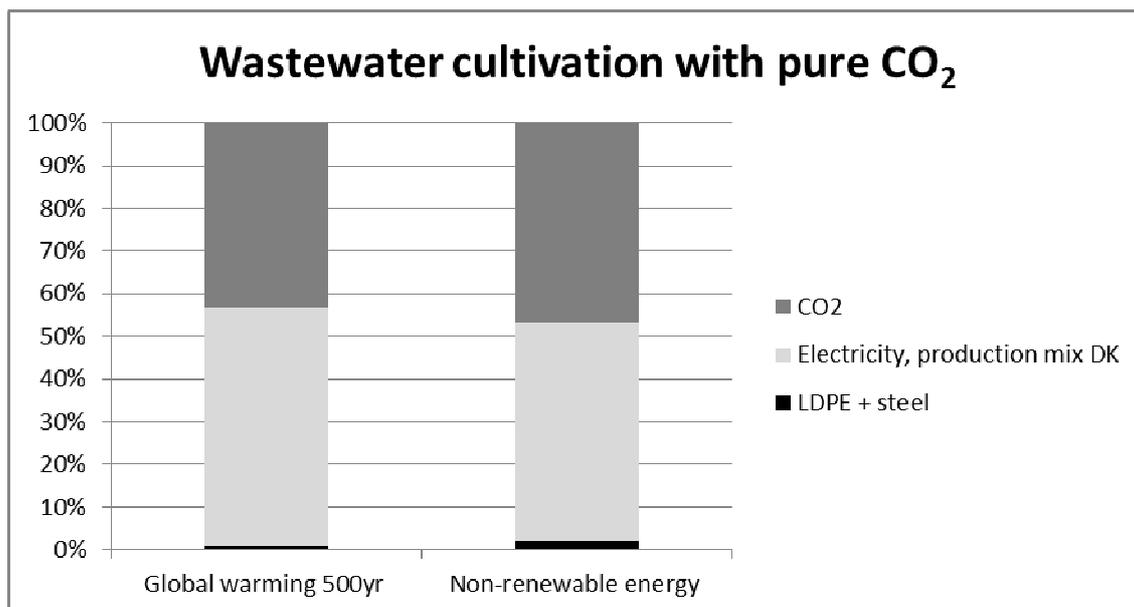
**Table 1.6: glycerol use phase**

## 2. LCIA: the relative contributions of each unit process in cultivation phase

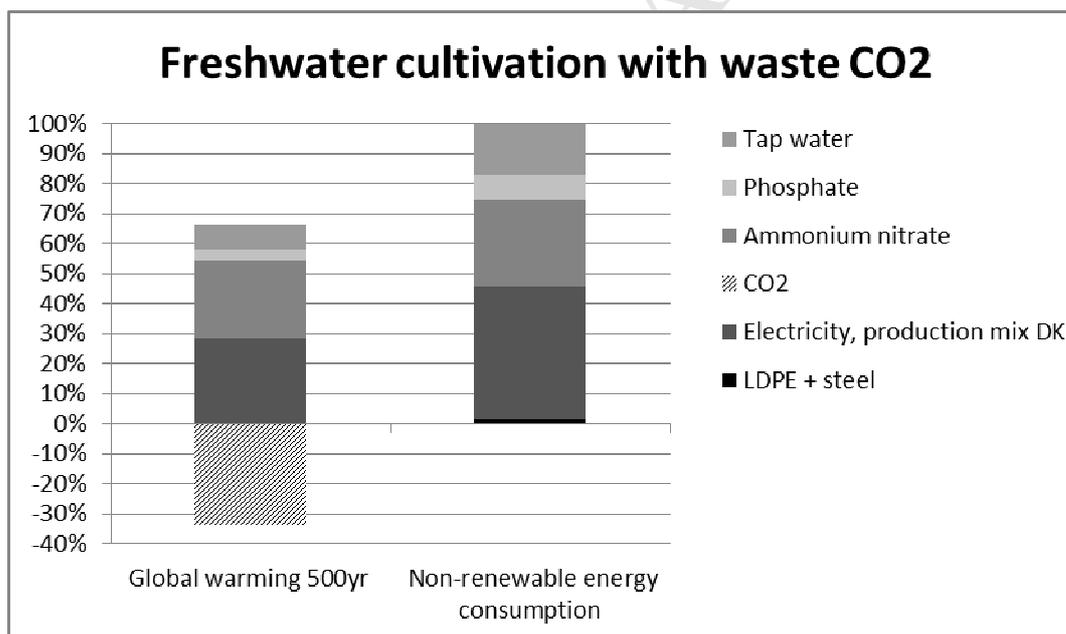
In this section, it is possible to observe the different processes used for cultivation and their relative weights to GWP and non renewable energy consumption for each case: freshwater cultivation and “pure CO<sub>2</sub>”, wastewater cultivation and “pure CO<sub>2</sub>”, freshwater cultivation and waste CO<sub>2</sub>, wastewater cultivation and waste CO<sub>2</sub>.



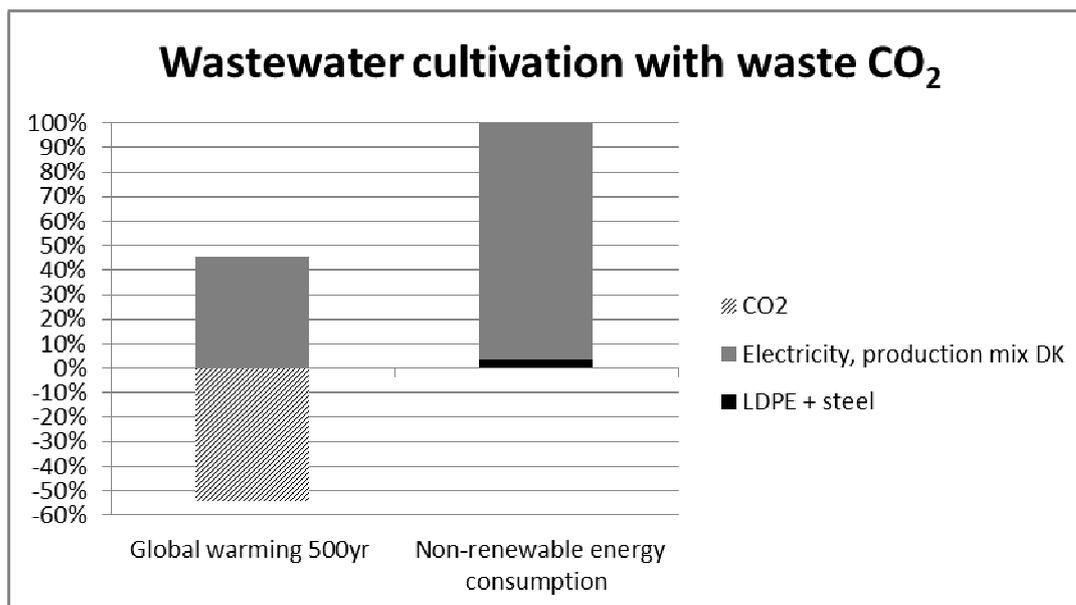
**Figure 2.1: contribution of each process unit in freshwater cultivation when “pure” CO<sub>2</sub> is used. In this case the unit processes considered are: tap water in which phosphate, ammonium nitrate and CO<sub>2</sub> are added, electricity for mixing and pumping CO<sub>2</sub> and LDPE for PBR construction**



**Figure 2.2:** contribution of each process unit in wastewater cultivation when “pure” CO<sub>2</sub> is used. In this case the unit processes considered are: wastewater (already enriched by phosphorus and nitrogen) in which CO<sub>2</sub> is added, electricity for mixing and pumping CO<sub>2</sub> and LDPE for PBR construction. In this case, the nutrients are not added to the water



**Figure 2.3:** contribution of each process unit in freshwater cultivation when waste CO<sub>2</sub> from a nearby cement industry is used for algal flow. In this case the unit processes considered are: tap water in which phosphate, ammonium nitrate and CO<sub>2</sub> are added, electricity for mixing and pumping CO<sub>2</sub> and LDPE for PBR construction. Since CO<sub>2</sub> is a waste flow, the negative contribution of CO<sub>2</sub> indicates that the flow does not take into account its production process



**Figure 2.4: contribution of each process unit in wastewater cultivation when waste CO<sub>2</sub> from a nearby cement industry is considered. In this case the unit processes considered are: wastewater (already enriched by phosphorus and nitrogen) in which CO<sub>2</sub> is added, electricity for mixing and pumping CO<sub>2</sub> and LDPE for PBR construction. In this case, the nutrients are not added to the water and since CO<sub>2</sub> is a waste flow, the negative contribution of CO<sub>2</sub> indicates that the flow does not take into account its production process**