



# Life cycle assessment on microalgal biodiesel production using a hybrid cultivation system



Victoria O. Adesanya<sup>a,\*</sup>, Erasmo Cadena<sup>b</sup>, Stuart A. Scott<sup>a</sup>, Alison G. Smith<sup>c</sup>

<sup>a</sup> Department of Engineering, University of Cambridge, Trumpington Street, Cambridge CB2 1PZ, United Kingdom

<sup>b</sup> Department of Hydraulic, Maritime and Environmental Engineering, Universitat Politècnica de Catalunya, BarcelonaTech, E-08034 Barcelona, Spain

<sup>c</sup> Department of Plant Sciences, University of Cambridge, Downing Site, Cambridge CB2 3EA, United Kingdom

## HIGHLIGHTS

- Life cycle assessment was performed on a putative algal biodiesel production plant.
- Hybrid cultivation system couples airlift tubular bioreactors with raceway ponds.
- Environmental impact of algal biodiesel was considerably lower than fossil diesel.
- Algal cultivation and drying of wet biomass were the two largest energy input.
- Sustainability of algal biodiesel depends on efficient utilization of co-products.

## ARTICLE INFO

### Article history:

Received 18 February 2014

Accepted 17 April 2014

Available online 26 April 2014

### Keywords:

Microalgae

Hybrid cultivation system

Biodiesel

Life cycle assessment

Environmental impact

## ABSTRACT

A life cycle assessment (LCA) was performed on a putative biodiesel production plant in which the freshwater alga *Chlorella vulgaris*, was grown using an existing system similar to a published commercial-scale hybrid cultivation. The hybrid system couples airlift tubular photobioreactors with raceway ponds in a two-stage process for high biomass growth and lipid accumulation. The results show that microalgal biodiesel production would have a significantly lower environmental impact than fossil-derived diesel. Based on the functional unit of 1 ton of biodiesel produced, the hybrid cultivation system and hypothetical downstream process (base case) would have 42% and 38% savings in global warming potential (GWP) and fossil-energy requirements (FER) when compared to fossil-derived diesel, respectively. Sensitivity analysis was performed to identify the most influential process parameters on the LCA results. The maximum reduction in GWP and FER was observed under mixotrophic growth conditions with savings of 76% and 75% when compared to conventional diesel, respectively.

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## 1. Introduction

Increasing global concerns on energy security and environmental deterioration owing to carbon emissions from combusting fossil fuels has led to growing research on sustainable and renewable alternatives to fossil-derived fuels. Biofuels (such as biodiesel, bioethanol, biohydrogen, biogasoline, and biogas) are considered sustainable and renewable sources of energy due to their relatively short processing time as well as the availability and continual replenishment of their feedstock (Schenk et al., 2008). Biofuels are nontoxic, biodegradable, low sulphur fuels that can reduce harmful emissions of greenhouse gases (GHGs), carbon monoxide, hydrocarbons and particulate matter (Mata et al., 2010). On a

life-cycle basis, biodiesel made from soybean oil has been reported to have a 78% reduction in net carbon dioxide emissions when compared to conventional diesel fuel (Tyson, 2001). Biodiesel blended directly with fossil-derived diesel has also been shown to have a substantial improvement on engine exhaust emissions. For example, Schumacher et al. (2001) reported that the combustion of biodiesel decreases carbon monoxide emissions by 46.7%, particulate matter emissions by 66.7% and unburned hydrocarbons by 45.2%.

On the other hand, biofuels produced from first generation feedstock (rapeseed, soybean, sunflower, wheat, corn) and second generation feedstock (switch grass, forestry waste and other lignocellulosic materials) have received criticism regarding their carbon mitigation potential and limited ability to achieve commercial targets for biofuels production (Mata et al., 2010). Areas of notable debates include food vs. fuel issues, requirement for arable land

\* Corresponding author. Tel.: +44 (0) 1223 332645.

E-mail address: [voa22@cam.ac.uk](mailto:voa22@cam.ac.uk) (V.O. Adesanya).

and freshwater, increase in deforestation, damages to biodiversity and significant amount of carbon dioxide released from the soil when considering land-use changes, especially the use of previously uncultivated land (Khoo et al., 2011). As an alternative to land-based crops, biodiesel from microalgae has received significant research interest due to their potential advantages over first- and second-generation feedstock (Chisti, 2007; Schenk et al., 2008).

Microalgae do not have to compete with food crops for arable land or other scarce agricultural inputs. Many microalgal strains grow rapidly and can be cultivated in open ponds or photobioreactors. Biomass productivity on a dry cell basis has been estimated to range from  $\sim 50$  to  $70 \text{ MT ha}^{-1} \text{ year}^{-1}$  when cultivated in high rate ponds (Sheehan et al., 1998; Carlsson et al., 2007) and  $\sim 150 \text{ MT ha}^{-1} \text{ year}^{-1}$  when grown in photobioreactors (Carlsson et al., 2007). This is in contrast with productivity of terrestrial crops:  $\sim 3 \text{ MT ha}^{-1} \text{ year}^{-1}$  for soybeans,  $\sim 9 \text{ MT ha}^{-1} \text{ year}^{-1}$  for corn, and  $\sim 10\text{--}13 \text{ MT ha}^{-1} \text{ year}^{-1}$  for switch grass (Perlack et al., 2005). Moreover, some microalgal strains are able to accumulate high lipid content (mostly between 20% and 50% per dry cell weight) which is more efficiently converted into biofuels than any other traditional biofuel-producing feedstock (Chisti, 2007). The potential yield of oil from microalgae has been estimated to be  $\sim 40 \text{ tons ha}^{-1}$  of oil on a large-scale, which is significantly greater than  $\sim 1.5 \text{ tons ha}^{-1}$  of oil from rapeseed grown in the U.K. (Rodolfi et al., 2009). Microalgal cultivation can also be integrated with wastewater treatment plants as they have the ability to utilize nutrients (e.g., nitrates and phosphates) from municipal waste water and agricultural waste (Sheehan et al., 1998). Furthermore, microalgae can utilize waste  $\text{CO}_2$  from power plants or other industrial sources, as a carbon source for biomass production.

Biofuels have the potential to be a carbon neutral alternative to fossil fuel, because the carbon dioxide fixed by photosynthesis during feedstock growth is later released upon combustion of the fuel. However, this carbon neutrality is not achieved in reality because emissions occur throughout the life cycle of the biofuel, starting from the cultivation of biomass, harvesting, drying, through to subsequent biofuel processing, storage of finished product, transportation, distribution and use (Mandil and Shihab-Eldin, 2010). The environmental burden associated with the biofuel production process is primarily due to energy requirements such as fuel and electricity to power machinery, heating during processing, land use change, as well as embodied energy to produce raw materials (e.g., fertilizers, methanol and construction materials). The overall net savings achieved by biofuels is dependent on the particular feedstock, production and management process, and the country and location of biofuel production (DfT, 2008). It is therefore important to evaluate the environmental impacts associated with biofuel production from different feedstocks.

LCA is a methodological tool used for quantifying the environmental impact and energy requirement of a product or service, from the extraction of raw materials through to its production, usage and end-of-life treatment, recycling and final disposal of wastes (i.e. cradle to grave). One of the key benefits of LCA stems from the fact that it provides information on the total environmental performance of a process which can be used as a decision-making tool in environmental management, monitoring and policy making. LCA also helps to identify energy and emission “bottle-necks”, i.e. life cycle stages of a process that are critical to the overall environmental burden and thus require further improvement (ISO, 2006a). The environmental impacts can be expressed in different categories such as global warming potential (GWP), fossil-energy requirement (FER), acidification potential, and eutrophication potential of water, which can be quantified by relating the emissions released by the process to a reference chemical. For example, GWP is expressed in terms of the equivalent mass of carbon dioxide emitted over a 100-years time horizon.

Several studies have investigated the sustainability and life cycle analysis of different aspects of the microalgae-to-fuel technology (Lardon et al., 2009; Stephenson et al., 2010; Clarens et al., 2010; Yang et al., 2011). A meta-analysis of 6 studies found that microalgal biodiesel is on par with terrestrial alternatives such as corn ethanol and soy biodiesel, and has the potential for lower GWP than fossil fuel (Liu et al., 2012). However, it was apparent that the actual outcome was heavily dependent on the modeling assumptions and system boundaries, in particular on the level of algal biomass and lipid content that could be achieved during the cultivation process. Stephenson et al. (2010) performed a comparative LCA study using either photobioreactors or raceway ponds in a two stage process, and found that while the production of microalgal-based biodiesel using raceway ponds was environmentally sustainable with a GWP  $\sim 80\%$  lower than fossil-derived diesel, that with airlift tubular photobioreactors resulted in a GWP significantly greater than the equivalent amount of fossil-derived diesel due to the high energy input for cultivation. Although the same functional unit (1 ton of biodiesel) was used in the study, the two cultivation systems investigated had different production capacities, with the raceway pond potentially harnessing the benefits of economies of scale, where a higher environmental burden is normalized by a large amount of final biodiesel product. This may not be a fair basis to conduct a comparative LCA study, and furthermore, there are many photobioreactor designs with improved energy use. The high environmental impact is probably not a useful indication of tubular photobioreactors on a large-scale and could be misleading during decision making for further consideration of microalgal biofuel production on a commercial-scale.

Interestingly, a recent comparative LCA study by Khoo et al. (2011) using a functional unit of 1 MJ biodiesel gave a different environmental impact result with the same data from Stephenson et al. (2010). The total life cycle energy demand and total life cycle net  $\text{CO}_2$  emitted for the open raceway ponds were estimated to be  $\sim 6.4 \text{ MJ/MJ}$  biodiesel and  $\sim 0.3 \text{ kg CO}_2/\text{MJ}$  biodiesel, respectively, while the values for the tubular bioreactors were  $\sim 0.9 \text{ MJ/MJ}$  biodiesel and  $\sim 0.02 \text{ kg CO}_2/\text{MJ}$  biodiesel, respectively. These findings show that the choice of functional unit, which provides the basis for calculating inputs and outputs, can also have a profound impact on the results of LCA studies.

In the current study, an LCA is conducted on biodiesel production from microalgae based on a so-called hybrid cultivation system which couples airlift tubular photobioreactors with raceway ponds, both having relatively the same production capacity, in a two-stage process (Huntley and Redalje, 2007). The hybrid cultivation system presents a synergistic effect which would harness the advantages of both photobioreactors (PBRs) and raceway ponds, whilst minimising inherent setbacks such as high production cost associated with photobioreactors and contamination in raceway ponds. Microalgae are grown continuously in photobioreactors under nutrient sufficient conditions for high biomass production, and then a portion is transferred to nutrient-deficient raceway ponds for high lipid accumulation. The downstream processes considered include harvesting, centrifugation, drying, cell disruption, extraction and transesterification, and GWP, FER and water footprint are the impact categories investigated. Several modifications to the production process and operation parameters were examined to determine key components of the process to which the LCA result is most sensitive.

## 2. Methods

### 2.1. Process definition and overall approach

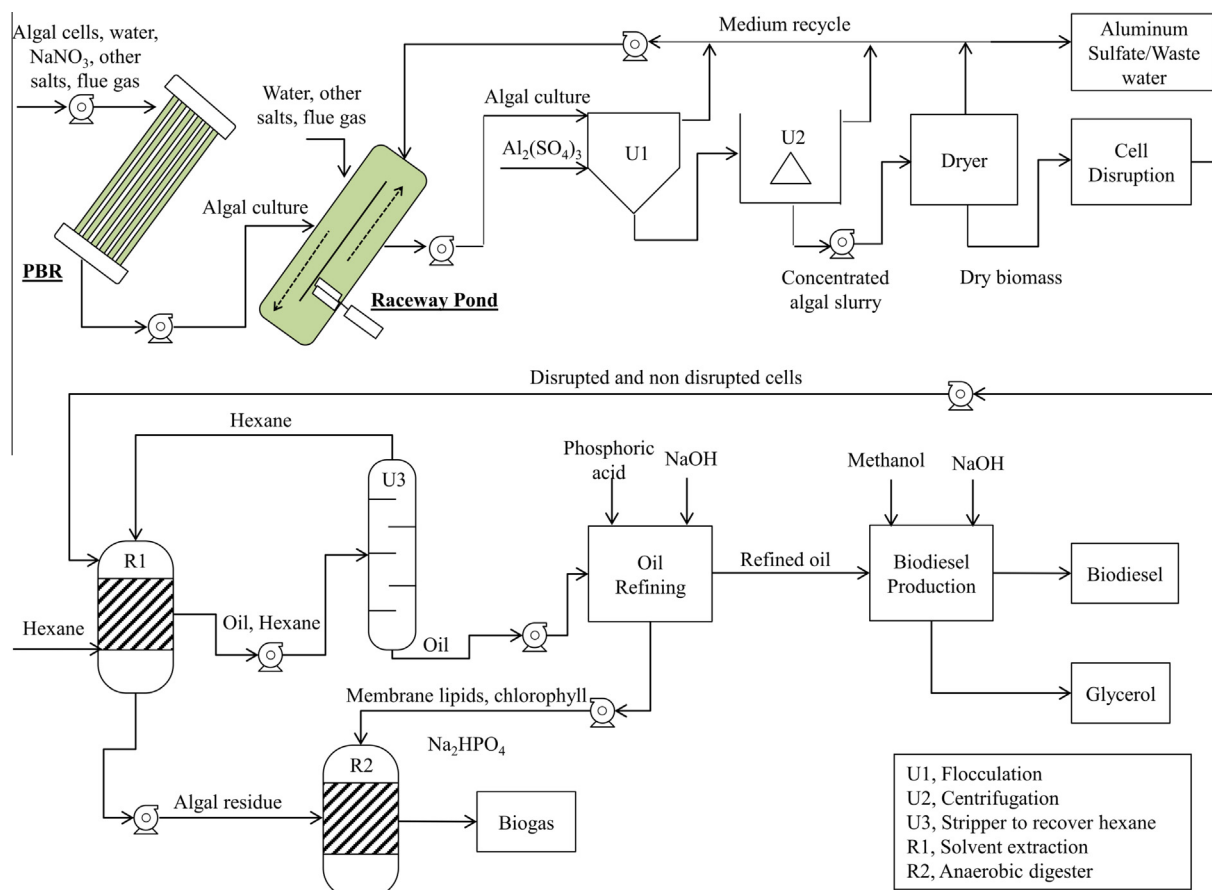
There is currently no commercial-scale production of biodiesel from microalgal feedstock, making it difficult to model a complete

- The green alga, *Chlorella vulgaris* is used as the model species due to its high growth rate and lipid content when grown under nitrogen stressed conditions (Rodolfi et al., 2009; Stephenson et al., 2010; Adesanya et al., 2014),
- A hybrid cultivation system consisting of airlift tubular reactors and raceway ponds is used to grow the microalgal biomass in a two-stage process (Huntley and Redalje, 2007),
- Wet microalgal biomass is harvested from the open pond after reaching high lipid content, dewatered to a slurry and further dried to produce dry biomass,
- Lipid is extracted from the dried microalgal biomass using solvents that are assumed to be fully recycled back into the process,
- The residual biomass after oil is extracted from the microalgal biomass is used to produce biogas in an anaerobic digester, which is used to generate electricity that can be used on-site in the production facility,
- Biodiesel, the main product, is produced via transesterification of the microalgal lipid with methanol. The reaction is catalyzed by the presence of the strong base, NaOH.

- Excess electricity required for the operation of the process plant would be supplied from the national energy network in the United Kingdom.

A process flow diagram for the base case scenario depicting the hybrid cultivation system and the downstream processing for microalgal biodiesel production is shown in [Fig.1](#). Different process scenarios were also explored through some modifications to the base case. A brief description of the base case scenario is presented in the subsections below. Further detail regarding the microalgal biodiesel process is provided in the [Supplementary material](#).

*C. vulgaris* cells are first grown in a commercial-scale (2 ha) hybrid cultivation system. This unique design consists of a dual cultivation process, which uses both photobioreactors and open raceway ponds in a two-stage method (Huntley and Redalje, 2007). The first stage consists of air-lift tubular photobioreactors where microalgal cells are grown to a high biomass concentration under nutrient-sufficient conditions. The second stage consists of typical raceway ponds where the cells are transferred into nitrogen-limited cultures for accumulation of lipid molecules. This two-stage cultivation approach is considered to be the most effective method for large-scale microalgal cultivation (Huntley and Redalje, 2007; Chisti, 2007). The design of the hybrid cultivation system has been engineered, built and successfully operated for continuous outdoor cultivation of another green alga, *Haematococcus pluvialis* for several years (Huntley and Redalje, 2007). The key



**Fig. 1.** Process flow diagram showing the material flow for microalgal cultivation in the hybrid system and the various stages in the downstream processing of algal slurry to biodiesel.

design parameters used for the airlift tubular photobioreactor and raceway ponds are presented in Table 1 and Table 2, respectively. Details of the design and construction materials used to estimate the associated burden of the process equipments during the LCA inventory stage can be found in the [Supplementary material](#).

Microalgae photosynthesize using carbon dioxide, which they normally obtain from the atmosphere. However, maximal productivity is achieved only after supplementation with additional carbon dioxide into the medium. Carbon dioxide would be supplied using flue gas with 12.5% carbon content from a natural gas-fired power plant. The hybrid system would run continuously, with the photosynthetic activity assumed to be 8 h/day at maximum rate (Stephenson et al., 2010) and operated as a batch process for the remaining time during the night. To ensure that all the microalgal cells receive the same amount of light, the grown culture from the bioreactor would be diluted by a factor of 2 before being transferred into the raceway pond. The culture would be left in the raceway pond until the maximum triacylglyceride (TAG) concentration has been attained, then the cells would be harvested and sent for further downstream processing. The nutrient requirement for microalgal growth under nutrient-sufficient conditions was estimated based on the cell composition. The average biochemical composition of microalgal biomass was assumed to have the molecular formula of  $\text{CH}_{1.83}\text{O}_{0.48}\text{N}_{0.11}\text{P}_{0.01}$  (Chisti (2007)). It was therefore assumed that the essential inorganic elements, nitrogen (N) and phosphorus (P), would be supplied in the form of ammonium nitrate (containing 34.5 wt% of nitrogen) and triple super phosphate fertilizer (containing 18 wt% phosphorus), respectively.

### 2.2.2. Downstream processing

The conversion of microalgal biomass to liquid transport fuels requires several downstream processes. A flowsheet for this hypothetical process is presented in Fig. 1. The process begins with harvesting and dewatering of the dilute culture broth in a two step process. Flocculation (U1), would be used to reduce the water by a factor of 25 using aluminium sulphate,  $\text{Al}_2(\text{SO}_4)_3$  as the flocculant. Further concentration of the settled sludge is achieved using a decanter centrifuge (U2), which causes an additional 5-fold reduction in the water content. The concentrated microalgal slurry exiting the centrifuge unit would be sent to a natural gas fired dryer where the cells would be dried to a 9% moisture content

**Table 1**  
Design parameters used for the Air-lift Tubular Photobioreactors.

Parameter	Units	Tubular reactor
Tube internal diameter	m	0.38
Tube external diameter	m	0.395
Tube length	m	510
Areal coverage of tube	$\text{m}^2$	246
Reactor volume	$\text{m}^3$	58.89
Mean liquid velocity	m/s	0.5
Reynolds number	–	190000
Residence time	days	2
Total air/gas for pump (STP)	$\text{m}^3/\text{day}$	3340
Useful power provided by air/gas	W	201.36
Overall efficiency of air-lift pump <sup>a</sup>	%	30.45
Dilution time <sup>b</sup>	h/day	8
Flue gas as carbon source (STP) <sup>c</sup>	$\text{m}^3/\text{day}$	135.42
Riser diameter	m	0.57
Riser height	m	4.15
Outlet biomass concentration	$\text{kg}/\text{m}^3$	1
Outlet algal flow rate <sup>c</sup>	$\text{m}^3/\text{day}$	29.39

<sup>a</sup> Fraction of electrical power supplied to the compressor, which is used to provide useful energy to the algal culture.

<sup>b</sup> Draw and fill protocol in hours per day when microalgae is continuously removed from the culture broth and fresh medium is supplied. The reactor is assumed to be held as batch systems during the remaining time.

<sup>c</sup> During dilution time only.

**Table 2**  
Design parameters used for the Open Raceway Pond.

Parameter	Units	Raceway
Pond depth	m	0.12
Pond length	m	288
Pond width	m	5.5
Hydraulic mean depth	m	0.1
Pond area	ha	0.32
Pond volume	$\text{m}^3$	392
Mean liquid velocity	m/s	0.3
Reynolds number	–	34378
Residence time	day	6
Dilution time <sup>a</sup>	h/day	8
Flue gas as carbon source (STP) <sup>b</sup>	$\text{m}^3/\text{day}$	190
Outlet biomass concentration	$\text{kg}/\text{m}^3$	1.67
Outlet algal flow rate	$\text{m}^3/\text{day}$	58

<sup>a</sup> Draw and fill protocol in hours per day when microalgae is continuously removed from the culture broth and fresh medium is supplied. The reactor is assumed to be held as batch systems during the remaining time.

<sup>b</sup> During dilution time only.

required for the solvent extraction step (Sander and Murthy, 2010). The effluent would be recycled immediately into the raceway ponds. After the harvesting and drying stage, the dry cells must be disrupted to break the cell walls and membranes and allow the TAG lipid to be recovered. Two methods of disrupting the cells were explored: (1) mechanical disruption using a high-pressure homogenizer or (2) enzymatic hydrolysis using “Cellic Ctec” enzyme.

Following disruption, the lysed cells would be pumped into an extraction column (R1) where the lipid would be extracted using a single-solvent (hexane) system. Lipid extraction is achieved by using a multistage countercurrent cascade consisting of five mixer-settlers (Benitez, 2009). The extract containing the solvent and TAG oil would be pumped into a stripper column (U3) for solvent recovery. The stripper column was modelled using UniSim® Design R400 and the non-random two liquid (NRTL) thermodynamic model was selected as the fluid property package for the simulation, as recommended for such calculations. Microalgal oil refining is yet to be practised on a commercial scale, hence, the process used to refine rapeseed oil for large scale biodiesel production in the U.K. (Stephenson et al., 2008) was assumed to be appropriate. The microalgal oil would be refined to remove contaminants such as chlorophyll and membrane lipid component from the cells.

The raffinate (lipid-depleted biomass) exiting the solvent extraction stage (R1) and the co-products from the oil refining stage would be sent to an anaerobic digestion facility (R2) to produce methane, which in turn would be converted to electricity and heat. The transesterification process of the refined microalgal oil into biodiesel was modelled based on the large-scale production of biodiesel from first generation feedstock in the U.K. (Stephenson et al., 2008). The transportation and distribution of the final biodiesel product was also similar to that of producing rapeseed biodiesel. Finally, this LCA study captures the GHG emissions associated with the combustion of the microalgal-based biodiesel in a compact-sized vehicle (see [Supplementary material](#) for details on the GHG emissions and allocation of co-product).

### 2.3. LCA framework

The LCA methodology used in this study followed the framework of the ISO 14040 and 14044 standards which involve the sequential phases of (i) goal and scope definition, (ii) inventory analysis, (iii) impact assessment, and (iv) interpretation and



reporting (ISO, 2006a,b). Further descriptions are provided in the following subsections.

### 2.3.1. Goal and scope definition

The specific goal of this LCA is to investigate the environmental impact for microalgal biodiesel production using a commercial-scale hybrid cultivation system, to which comparisons are made against fossil-derived diesel. The functional unit was defined as 1 ton of biodiesel produced, which has been blended to a specific volume fraction with fossil-derived diesel, delivered to a filling station in the UK and later combusted in a typical compact-sized car engine (Stephenson et al., 2010).

The process system boundary was defined to include all the fundamental processes directly used in the production of microalgal biodiesel (microalgal cultivation, harvesting, centrifugation, oil extraction and biodiesel production) and combustion of the biodiesel in a typical car engine. The burden associated with the manufacture of construction materials for all the process equipments is also included within the system boundary. Fig. 2 shows the process flowchart consisting of all the major unit processes linked to one another by material flows of the inputs and outputs of each unit process within the process system boundary.

### 2.3.2. Inventory analysis

A summary of data sources and information obtained for process parameters and energy requirements are presented in Table 3. Substantial data for microalgal growth was obtained from academic resources and our laboratory experiments of growing *C. vulgaris* (Stephenson et al., 2010; Adesanya et al., 2014), which were modelled into a process flow sheet. These data were analytically evaluated in order to quantify and compile all the input and output flows for each stage within the process chain. The flows included material resources, energy inputs, products, co-products, emissions and wastes, which were normalized to the functional unit. In the event where sufficient data was not available due to absence of large-scale microalgal biodiesel production, some assumptions were made based on commercial-scale biodiesel production from first generation feedstock in the U.K. as well as existing industrial technologies. The system was modelled in a Microsoft Excel spreadsheet where the process data was organised for ease of numerous calculation scenarios and model sensitivity analyses. Data used in this LCA study that required distinction such as on electrical power and construction materials were made specific to the U.K. Further details on the data source and assumptions used for the LCA modelling can be found in the [Supplementary material](#).

### 2.3.3. Impact assessment

The environmental impact associated with each of the input, output and emission in the LCA inventory was evaluated using the openLCA version 1.3 software and different LCA databases (Ecoinvent v2.2 (Ecoinvent Centre), ELCD (Europe) and NREL (U.S.)). The CML 2001 method was used to conduct the life cycle impact assessment. In this study, the set of impact categories reported are global warming potential, GWP (kg of CO<sub>2</sub>-eq/ton of biodiesel) over a time horizon of 100 years, fossil-energy requirement, FER (GJ/ton of biodiesel), and water footprint (m<sup>3</sup>/ton of biodiesel) used to produce 1 ton of biodiesel.

### 2.3.4. Interpretation and reporting

The robustness of the impact assessment results for the base case scenario was validated by sensitivity analysis to identify the key parameters which had the largest effect on the LCA results. The LCA results were reported using the principles and guidelines reported in the ISO standards (ISO, 2006a,b).

## 2.4. Modification of the base case process

In order to understand how parameter variations affect the results and to identify the parameters and unit processes that significantly influence the LCA result, sensitivity analysis was performed with several modifications to the base case scenario. The variations investigated include: (1) mixotrophic microalgal growth in airlift tubular reactor; (2) areal TAG productivity of cultivation system; (3) flow velocity of the culture within the airlift tubular reactor; (4) liquid flow velocity of culture in the open raceway pond; (5) discharging the effluents to a wastewater treatment plant rather than recycling into the cultivation system; (6) cell disruption by enzymatic hydrolysis rather than mechanical homogenization; and (7) recovery of lipid content from disrupted microalgal cells.

## 2.5. Comparison with fossil-derived diesel

The results from this study were compared with the energy requirement and CO<sub>2</sub> emissions from fossil-derived diesel (sulphur content of 0.1% by mass) using available data from the literature. The comparison is performed on a net energy content basis for both biodiesel and fossil diesel. It was assumed that the fuel energy efficiency (i.e. distance travelled per MJ of fuel combusted) is the same for both biodiesel and fossil diesel, such that 1 MJ of biodiesel displaces 1 MJ of fossil diesel. Taking the lower calorific value of diesel to be 43.1 MJ/kg, the reference value for the fossil-energy requirement of diesel was assumed to be 1.16 GJ/GJ (equivalent to 50 GJ/ton of diesel) and that for GWP was assumed to be 86 kg CO<sub>2</sub>-eq/GJ (3707 kg CO<sub>2</sub>-eq/ton of diesel) (DfT, 2008). The direct savings (as a percentage) on the environmental burden using biodiesel are calculated using the following equation:

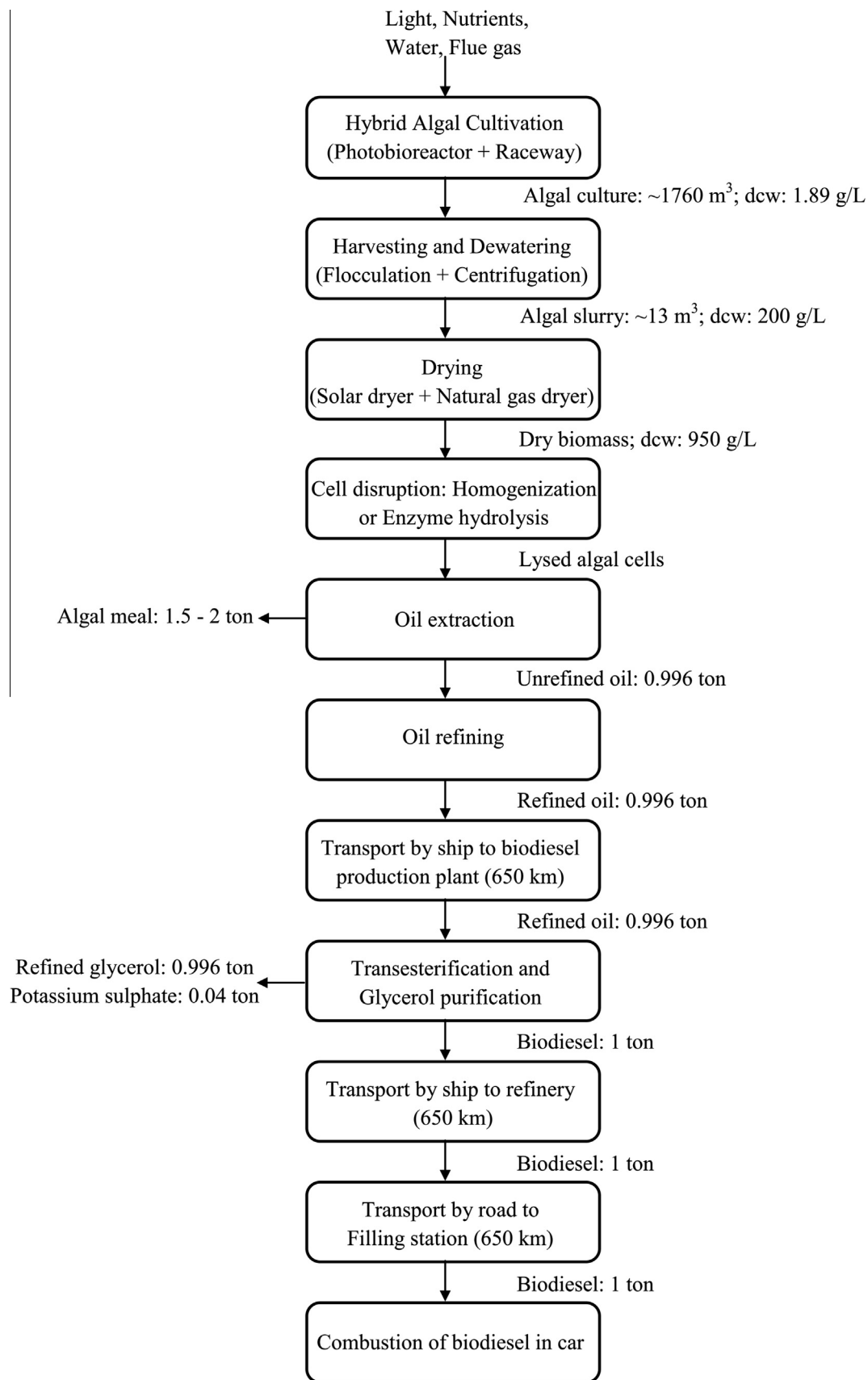
$$\text{Savings} = \left( \frac{\text{FD} - \text{BD}}{\text{FD}} \right) \times 100 \quad (1)$$

Where, FD is the environmental burden of the fossil diesel and BD is the environmental burden of the microalgae-to-biodiesel process.

## 3. Results and discussions

### 3.1. Environmental performance of the base case

The global warming potential (GWP) and fossil-energy requirement (FER) associated with the production of 1 ton of biodiesel from *C. vulgaris*, grown in a hybrid cultivation system and processed via the base case downstream stages, are presented in Fig. 3a and b, respectively. As observed in other studies (Liu et al., 2012), the burden of microalgal cultivation and drying of the slurry is found to contribute to more than 84% of the total impact. The environmental impact associated with the electricity and heat requirement for all the LCA stages are shown, as well as the offset resulting from the electricity and heat production from the methane generated by anaerobic digestion of the residual microalgal biomass. On an energy basis, the LCA result indicates that the GWP and FER of biodiesel production from the cultivation of microalgae in a hybrid system give savings of 42% and 38% when compared to fossil-derived diesel, respectively. Although, the GWP and FER savings for this hybrid system are lower than the value reported by Stephenson et al. (2010) for raceway ponds (estimated to be 78% and 85%, respectively), this former study did not consider drying, which is an energy intensive process (~40% of total burden) and also required to dry cells for single solvent extraction. Whilst extracting the TAG component from wet biomass is a practical option for reducing the overall energy demand of the microalgal biodiesel process, achieving high TAG yield from



**Fig. 2.** Process value chain for algal biodiesel production showing all the principal stages and material flows involved in the production of 1 ton of biodiesel that has been blended with fossil-derived fuel to a specific volume fraction, delivered to a filling station in the U.K. and combusted in a compact-sized car engine. dcw = dry cell weight.

**Table 3**

Summary of data sources and process parameters used in this LCA study.

Unit Process	Data source	Data gathered from source and assumptions
Algal growth	Rodolfi et al. (2009), Huntley and Redalje (2007), Adesanya et al. (2014), Acien Fernández et al. (2001), Weissman and Goebel (1987)	Algal concentration and productivity. Design specification parameters for hybrid cultivation system. Principles of fluid mechanics, gas–liquid mass transfer, irradiance-dependent algal cell growth in airlift tubular photobioreactor. Paddle wheel, carbonation sump and pond design. Concrete blocks would have a lifespan of 20 years with dimensions of $0.44 \times 0.22 \times 0.215$ m (CBA, 2007). PVC lining thickness of $0.75 \times 10^{-3}$ m and having a lifespan of 5 years is used
Flocculation	Weissman and Goebel (1987), Stephenson et al. (2010)	Electrical requirements and operating parameters. Aluminium sulphate added to culture at a dosage of 0.15 g/l in a flocc tank with agitation power of 100 W/m <sup>3</sup> for a residence time of 20 min. Algal slurry from tank is pumped into a 3 m deep circular settling pond with PVC lining, having a settling velocity of $\sim 1.5$ m/s. Algal sediment discharged into a central hopper using a rotating flight scraper with power requirement of 0.8 W/m <sup>2</sup> of pond area
Centrifugation	Molina et al. (2003)	Unit process equipment and operating parameters. Each decanter centrifuge would treat 2000 L/h slurry. 5-fold cell concentration is achieved reaching a final slurry density of 220 g/L. Electrical requirement is 0.28 MJ/L of algal slurry entering the centrifuge. Cell recovery efficiency is 95 wt% algal cells, leaving 5 wt% loss of algal cells
Drying	Sander and Murthy (2010)	Electrical requirement and emissions of natural gas dryers. 3556 kJ/kg of water removed is required
Cell disruption	GEA Process Engineering (2009), Shahindi (1991), Stephenson et al. (2010)	GEA Ariete homogenizer, NS-3037. Slurry capacity is 2000 L/h when operated at a pressure of 600 bar and requires cooling water at a rate of 90 L/h. Process unit constructed with 2.1 tons of stainless steel has an AC motor requiring a power input of 37 kW per pass. Electrical requirement calculated as $\sim 67$ kJ/l of algal slurry. Two-pass approach is used to achieve 96% cell disruption. Enzyme hydrolysis process information based on rapeseed feedstock. Novozymes A/S, “Cellic Ctec” is employed at a dosage of 0.02 kg/kg of dry cell weight. Continuous stirred tank reactor (CSTR), of residence time 4 h, is used for enzyme treatment and agitated at 1 MW/L with motor efficiency of 90%. Cell disruption yield is assumed to be 80% disrupted algal cells
Oil extraction	Benitez (2009)	Design and power requirement of a counter-current cascade of five steel mixer-settlers. Impeller rotation rate and power input is $\sim 5.3$ Hz and $\sim 3.3$ MW/L of the mixer volume. Slurry viscosity
Biodiesel production	Stephenson et al. (2008)	Large-scale biodiesel production plant in the UK with production capacity 250000 tons of biodiesel per annum. Transportation and distribution of raw materials and final product
Solvent recovery	UniSim® Design R400	Stripper column modelled using UniSim® Design R400. Column pressure is 1 bar with hexane recovery of 99%. Reboiler heat requirement is $\sim 2.24$ kJ/kg TAG entering the distillation column
Anaerobic digestion	Sialve et al. (2009)	Methane yields from protein, lipid and carbohydrate, COD removal and nutrient requirement of a CSTR digester. Agitation power is $\sim 10$ W/m <sup>3</sup> . Waste stream is sent to wastewater treatment plant

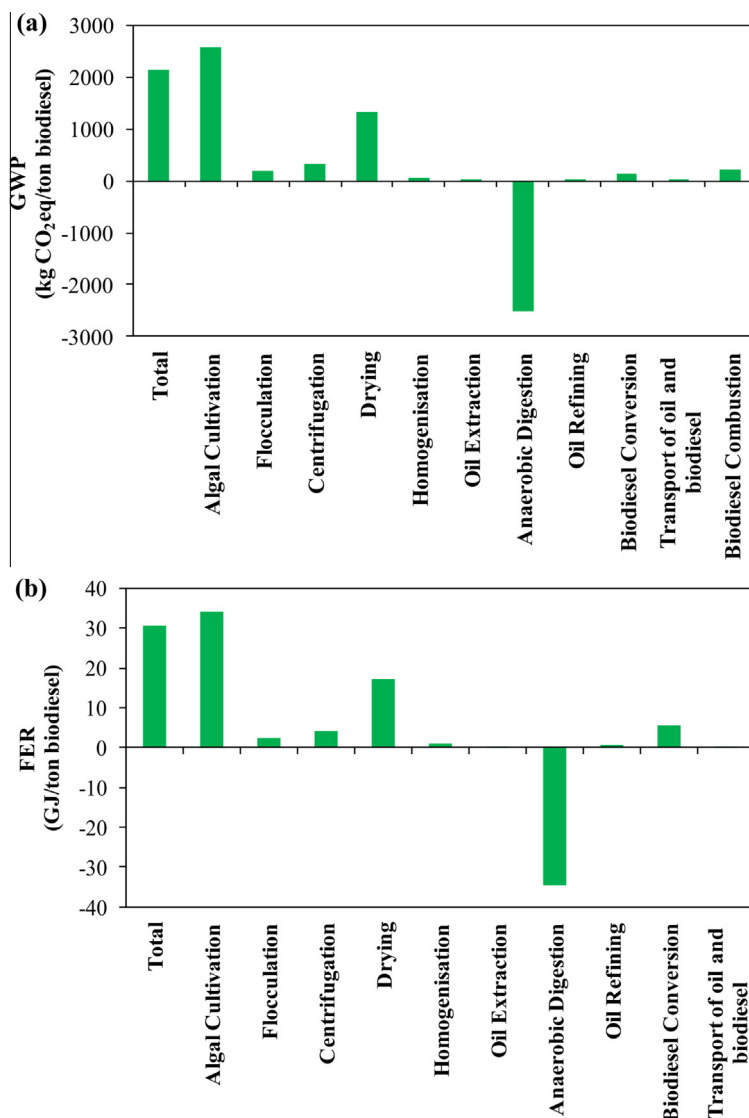
the wet extraction process would require a co-solvent mixture such as heptane/isopropanol, hexane/methanol, chloroform/methanol (Khoo et al., 2011; Horst et al., 2012). The presence of a co-solvent further adds to the complexity of solvent recovery and recycling and also creates significant problems with waste disposal on an industrial scale.

It is important to state that the 42% GWP saving takes into account the carbon credit for the use of flue gas in the microalgal cultivation stage. This carbon credit offsets the environmental burden associated with the combustion of biodiesel in a compact-sized car engine as observed by the small bar in Fig. 3a. The flue gas would otherwise have been released to the atmosphere if it were not used for microalgal cultivation. The LCA results show that biodiesel production from *C. vulgaris* cultivated in the hybrid system under investigation would have a lower GWP and FER than many first generation biodiesels. For instance, biodiesel produced from rapeseed, soybean, palm and sunflower has been estimated to have net savings ranging from 20% to 38% GWP when compared to conventional diesel (RFA, 2011). In addition, the results demonstrate that microalgal biodiesel production gives higher GHG savings than other biofuels. For instance, LCA studies on bioethanol production from corn and wheat in the U.K. has been reported to have a GWP 28% lower than fossil derived gasoline, excluding the impact of land-use change (Gallagher, 2008).

### 3.2. Major contributions to the microalgal cultivation stage

It can be observed from Fig. 3 that the cultivation of the microalgal biomass in the hybrid system contributes the most to the GWP and FER of the biodiesel production using the base case method. A breakdown of all the main components contributing to the GWP and FER associated with the cultivation of *C. vulgaris* in the hybrid system is illustrated in Fig. 4. The plots show that the burden associated with the airlift tubular bioreactor and raceway pond in a hybrid setup are relatively of the same order of magnitude. This is in contrast to the results presented in Stephenson et al. (2010), where the burden of the tubular photobioreactor was estimated to be 10 times more than that for the raceway open pond, with the two production systems having a combined production capacity of 1.83 and 3645 m<sup>3</sup>, respectively. This LCA study, however, makes a fair comparison because it maintains relatively similar production capacity for the two types of systems.

It is observed that the electrical power requirement and the manufacture of the construction materials for both the airlift tubular photobioreactor and raceway pond are the input factors that contribute significantly to the overall environmental burden. The electricity demand for the cultivation stage accounts for 62% and 60% of the total GWP and total fossil energy demand, respectively. The direct electricity demand for the base case



**Fig. 3.** Environmental performance for each unit process using the base case to produce 1 ton of biodiesel from *C. vulgaris* grown in the hybrid cultivation system: (a) GWP and (b) FER.

scenario was estimated to be 23.2 GJ/ton of biodiesel (excluding the indirect energy required for electricity production), which is more than the electricity generated by combusting the methane produced via anaerobic digestion of the residual microalgal biomass (~14.8 GJ/ton of biodiesel). Hence, the results show that the process of biodiesel production from microalgae cultivated in the hybrid system under investigation would require an electricity supply of 8.4 GJ/ton of biodiesel from the national grid network.

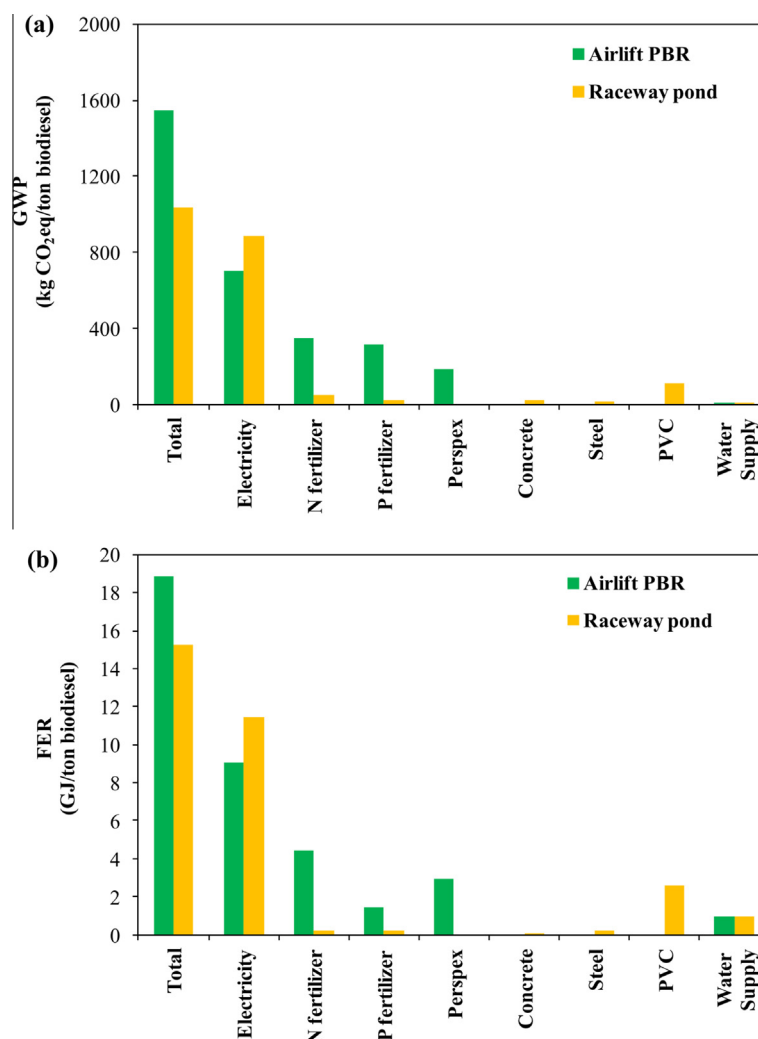
The nutrient requirement for microalgal growth was calculated to contribute 28% and 18% of the total GWP and FER for the hybrid cultivation system, respectively. Figs. 4 and 5 illustrate that a large portion of the burden from the supply of nutrients is associated with the tubular photobioreactor where algal cells are grown under nutrient sufficient conditions. The nutrient supply in the raceway pond was found to be less significant because the microalgae are grown under nitrogen deficient conditions for lipid accumulation. The environmental burden associated with the manufacture of the construction material for airlift tubular photobioreactor (perspex tubing) was estimated to contribute 7.2% and 8.5% to the total GWP and FER of the cultivation stage, respectively, while the burden from the manufacture of polyvinyl chloride (PVC)

lining used to build the raceway pond was found to represent 4.2% and 7.5% of the GWP and FER, respectively.

### 3.3. Water usage and water footprint

The water usage of microalgal biodiesel production for the base case scenario when cultivating microalgae in the hybrid system with recycling of the harvested spent medium was estimated to be 16.5 m<sup>3</sup>/ton biodiesel. When supernatants from the dewatering process cannot be reused for microalgal cultivation due to contamination or the accumulation of toxic products that could inhibit growth, the spent medium would be sent to a wastewater treatment plant. About 89% of the water would be discharged after harvest, while the rest would be lost by either pond evaporation or drying during cultivation. The amount of water discharged in this study is similar to the 84% water usage reduction reported by Yang et al. (2011) when recycling harvested water. In this case, the water requirement for microalgal growth would be significantly more than the base case because of the large volume of harvested microalgae, with the hybrid system having a water footprint of approximately 1700 m<sup>3</sup>/ton biodiesel. The overall GWP and FER for microalgal biodiesel production resulting from the higher water





**Fig. 4.** Contributions to the environmental burden for the base case cultivation of *C. vulgaris* in the hybrid system consisting of airlift tubular reactors and raceway ponds (a) GWP and (b) FER.

requirement and treatment of large volumes of wastewater when the harvested spent medium cannot be recycled was observed to increase significantly, giving a reduced GWP and FER savings of 22% and 29%, respectively when compared to fossil-derived diesel as shown in Table 4. It is important to state that the water usage at different stages of the downstream process does not vary with recycling of harvested water.

### 3.4. Modifications of the base case

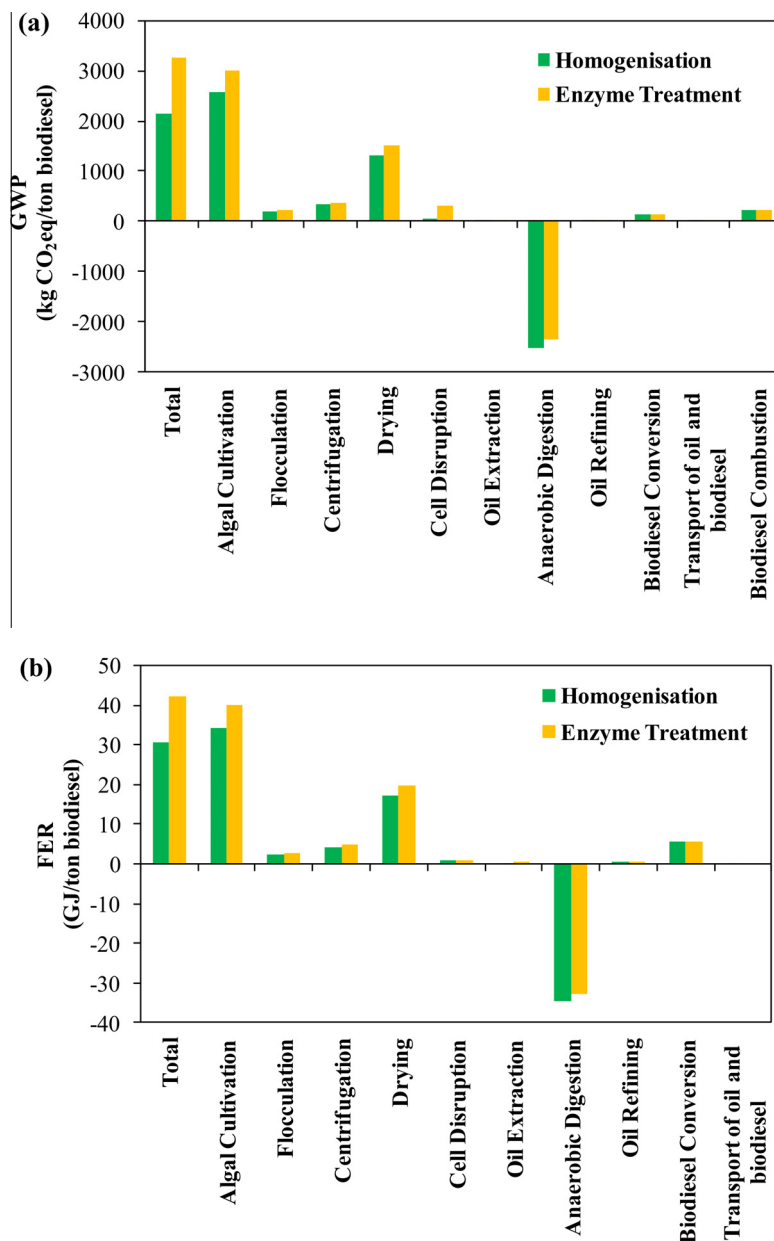
The environmental impact of large scale microalgal biodiesel production has an inherent degree of uncertainty due to lack of industrial data. Having described the base case, several parameters for the hypothetical biodiesel plant were varied so as to investigate their respective influences on the LCA results. The sensitivity of the LCA results to several modifications of the base case is presented in Table 4. It can be observed that the results are highly sensitive to some of the parameters considered in this study.

#### 3.4.1. Sensitivity to mode of cultivation

There are three major metabolic pathways that can be utilized by microalgae depending on light and carbon sources: photoautotrophy, heterotrophy and mixotrophy (Mata et al., 2010). Photoautotrophic microalgal cultivation was assumed for the base case scenario, however this approach is limited by low biomass density

on a large scale because of limited light transmission, especially in dense cultures. Mixotrophic cultivation can significantly boost biomass and lipid production of microalgae in large scale systems due to the synergistic effect of photosynthesis and heterotrophy (Wu et al., 2012). Bhatnagar et al. (2011) found that mixotrophic growth of *Chlorella minutissima* with 10 g/l glucose resulted in 3–10 times more biomass production when compared to that produced under photoautotrophic growth conditions. Our laboratory experience of growing *C. vulgaris* in mixotrophic cultures with 0.5 g/l glucose as the organic carbon source also achieved ~2-fold increase in biomass productivity when compared to photoautotrophic cultures (Adesanya et al., 2014).

When the analysis was modified to mixotrophic cultivation of *C. vulgaris* using glucose as an organic carbon source, significant improvement in terms of environmental performance of the microalgal biodiesel production process was observed, leading to net savings in GWP and FER approaching 76% and 75%, respectively, relative to fossil derived diesel. The low environmental impact can be attributed to the fact that highly dense cultures enhances flocculation as there is significant cell-to-cell interactions in highly concentrated suspensions thereby, eliminating the need for secondary biomass recovery via centrifugation which is energy intensive and expensive (Molina et al., 2003). Also, increased biomass and lipid production means more biodiesel could be produced with the same volume of cultivation system. It was found that under



**Fig. 5.** Environmental burden of biodiesel production from *C. vulgaris* using two different cell disruption methods: homogenisation (base case) and enzyme treatment. (a) GWP and (b) FER.

mixotrophic cultivation the electricity demand of the biodiesel production plant was reduced to  $\sim 17.4$  GJ/ton of biodiesel, which is slightly below the electricity generated at the anaerobic digestion stage. This LCA shows that biodiesel production from mixotrophic cultivation of microalgae in the hybrid system under investigation is close to being energetically self-sufficient, requiring an electricity supply of 2.8 GJ/ton of biodiesel from the national grid network.

Although, mixotrophic cultivation has the potential to fulfil the needs of sustainable microalgal biofuel processing on a large scale, detailed economic analysis is required when using glucose as the organic carbon source to ensure that the cost of the final biodiesel product remains competitive with conventional diesel. Furthermore, research into low-cost carbon substrates from industrial and agricultural wastes, municipal wastewater, effluents from anaerobic digestion, as well as crude glycerol from the biodiesel production process is evidently required if mixotrophic cultivation

method is to be used on a commercial scale. Moreover, the inclusion of an organic carbon source in the cultivation steps is likely to increase considerably the potential for contamination by adventitious microbes, such as heterotrophic bacteria and fungi, which would be likely to reduce yields. Production losses under these circumstances may well offset any potential benefits.

#### 3.4.2. Sensitivity to areal TAG productivity

The oil content of microalgae is one of the most important parameters that establish the potential of microalgal-based biodiesel production to meet global demand for transport fuels (Chisti, 2007). A TAG productivity of 40 tons  $\text{ha}^{-1} \text{year}^{-1}$  was used for the base case process from the research of Rodolfi et al. (2009). However, there are several published projections that estimate future yields of microalgal lipid between 80 and 90 tons  $\text{ha}^{-1} \text{year}^{-1}$  (Chisti, 2007; Schenk et al., 2008). It can be observed from Table 4 that TAG productivity has a positive effect on the environmental

**Table 4**Sensitivity of GWP and FER for biodiesel production from *C. vulgaris* grown in a hybrid system of cultivation considering modifications to the base case<sup>a</sup>.

Scenario	GWP		FER	
	kg of CO <sub>2</sub> -eq/ton of biodiesel	Savings (%) <sup>b</sup>	GJ/ton of biodiesel	Savings (%) <sup>b</sup>
Base case				
Hybrid system	2137	42	31	38
Modifications to the base case				
Mode of cultivation (base = photoautotrophic)				
Mixotrophic	896	76	12	74
Areal TAG productivity (base = 40 tons ha <sup>-1</sup> year <sup>-1</sup> )				
80 tons ha <sup>-1</sup> year <sup>-1</sup>	1721	54	24	50
20 tons ha <sup>-1</sup> year <sup>-1</sup>	2929	21	41	16
10 tons ha <sup>-1</sup> year <sup>-1</sup>	4515	–22	64	–28
Culture velocity in airlift bioreactor (base = 0.5 m/s)				
0.35 m/s	1825	51	27	47
0.75	3062	17	42	15
Culture velocity in raceway pond (base = 0.3 m/s)				
0.15 m/s	1565	58	23	54
0.45 m/s	3685	0.6	51	–1.5
Water usage (base = recycle spent medium to cultivation)				
Spent medium to WWT <sup>c</sup>	2890	22	35.5	29
Cell disruption (base = homogenisation)				
Enzyme treatment <sup>d</sup>	3249	12	42	15
Enzyme treatment <sup>e</sup>	3106	16	41	17
Recovery of lipid (base = 99%)				
50%	–1732	146	–19	138

<sup>a</sup> The environmental performance for the base case is presented at the top of the table, and the values calculated by changing each of the subsequent parameters, whilst keeping all other factors constant as in the base case) are shown below.

<sup>b</sup> Savings when compared to fossil-derived diesel on a net energy basis.

<sup>c</sup> WWT is wastewater treatment.

<sup>d</sup> Enzyme treatment based on Shahindi (1991) and Novoenzymes (2009).

<sup>e</sup> Enzyme treatment based on Horst et al. (2012).

burden of microalgal biodiesel production i.e. increasing the productivity of microalgal oil reduces the burdens and *vice versa*. The LCA results indicate that when the TAG productivity is doubled to 80 tons ha<sup>-1</sup> year<sup>-1</sup>, the GWP and FER were estimated to be 54% and 50% lower than fossil derived diesel, respectively. For a TAG yield of 20 tons ha<sup>-1</sup> year<sup>-1</sup>, the environmental burden was increased giving a net saving of GWP and FER of 21% and 16%, respectively, and at the lowest oil yield of 10 tons ha<sup>-1</sup> year<sup>-1</sup>, biodiesel production from microalgal feedstock has a higher environmental impact in terms of both GWP and FER when compared to fossil derived diesel.

### 3.4.3. Sensitivity to culture velocity in cultivation system

Mixing is important in microalgal cultivation to prevent sedimentation of cells and enhance gas and liquid mass transfer. Assuming that biomass and TAG productivity are constant in the solar collector and raceway pond, respectively, it is observed from Table 4 that the LCA results is highly sensitive to changes in the liquid velocity for the cultivation facilities. The performance of an airlift tubular photobioreactors with varying liquid flow velocities has been investigated by Acien Fernández et al. (2001). The authors reported that growing cultures of the diatom, *Phaeodactylum tricornutum* using velocities of 0.5 and 0.35 m/s gave similar biomass productivities, while the culture quickly died at a lower velocity of 0.15 m/s, owing to the toxic effects of the accumulation of oxygen from photosynthesis, and longer residence time of microalgal cells along the length of the solar tubular loop. When the liquid flow velocity in the tubular bioreactor is reduced from 0.5 m/s (base case) to 0.35 m/s, the direct electrical power requirement for the biodiesel production process reduces to 21.3 GJ/ton of biodiesel, giving an overall reduction in GHG emission and FER by 51% and 47%, respectively. On the other hand, when the circulation

velocity of culture in the raceway pond is reduced from 0.3 m/s (base case) to 0.15 m/s, the electricity demand falls to 19.8 GJ/ton of biodiesel, resulting in an overall GWP and FER savings of 58% and 54% when compared to fossil derived diesel, respectively.

### 3.4.4. Sensitivity to the method of cell disruption

In order to access the intracellular lipid content within microalgal cells, mechanical cell disruption via homogenization was used for the base case. The model species *C. vulgaris*, has a recalcitrant cellulosic cell wall, therefore the use of the cellulase enzyme, Cellic Ctec, was also investigated in degrading the microalgal cell wall. When the analyses was modified to use enzyme treatment method rather than homogenization, the savings for both GHG emissions and FER was reduced to 12% and 15%, respectively when compared to fossil derived diesel as shown in Table 4. The major contributors to the environmental burden of the enzyme treatment process are enzyme loading and heat requirements representing 48% and 34%, respectively. The impact associated with the production of the enzymes was estimated to be ~280 kg CO<sub>2</sub>-eq/ton biodiesel.

Fig. 5 shows the comparison of the environmental burden associated with each stage of the microalgal biodiesel process for the two methods of cell disruption. It can be observed that the environmental impact associated with all the sub-processes before the cell disruption stages (microalgal cultivation, flocculation, centrifugation and drying) would increase if enzymes are used to degrade the microalgal cell walls. This result is expected as it was assumed that only 80 wt% of the cells would be disrupted using enzyme treatment as opposed to the 96 wt% of disrupted cells obtained using the homogenization process. This reduces the amount of TAG produced after oil extraction when processing the same volume of microalgal slurry. Furthermore, the presence of intact cells reduces the biodegradability of the microalgal

residue and methane conversion yield (Sialve et al., 2009), therefore, less electricity is generated per ton of biodiesel produced.

The processing condition for enzymatic hydrolysis of microalgal cell wall based on the recent publication by Horst et al. (2012) was also investigated. The authors reported that an enzyme loading of 0.01 mg/mg of dry cell weight (half the enzyme loading for the base case) was sufficient to degrade the cell walls of *P. tricornutum* using crude papain and for an optimal incubation time of 2 h. The LCA results when using these mild process conditions show a little improvement in GHG emissions owing to the reduced enzyme dosage requirement, however the burden from the heat demand for the enzymatic treatment makes this process less sustainable than homogenization. The estimated savings on GWP and FER was estimated to be 16% and 17% when compared to conventional diesel, respectively. Since enzyme dosage requirement is significantly dependent on feedstock type and optimal processing conditions, pilot-scale trial research is needed to investigate these assumptions.

#### 3.4.5. Sensitivity to recovery of lipid

The recovery of the lipid content from the disrupted microalgal cells has a significant effect on the environmental performance of the microalgal biodiesel production process. On a mass basis, if the lipid recovery after solvent extraction was assumed to decrease from 99% (base case) to 50%, the amount of biodiesel produced per ha would decrease by a factor of  $\sim 2$ ; however, GWP and FER savings would significantly increase to 146% and 138%, respectively. This somewhat counter intuitive finding arises because excess lipid left in the residual biomass would ultimately be sent to the anaerobic digester, causing more methane to be produced resulting in the production of more electricity (10.9 GJ of electrical power exported to the national grid per ton of biodiesel produced). This surplus electricity generated from the biodiesel production process offsets the overall environmental burden. Sialve et al. (2009) reported the specific methane yield (expressed in litres of CH<sub>4</sub> per gram of volatile solids, VS) for lipid, protein and carbohydrate as 1.014, 0.851 and 0.415 L CH<sub>4</sub> g VS<sup>-1</sup>, respectively. This implies that the higher the microalgal lipid content, the higher the potential methane yields and subsequently more electricity generation.

## 4. Conclusion

This LCA study quantifies the environmental impact of microalgal-based biodiesel production using a hybrid cultivation system. For 1 ton of microalgal biodiesel produced via the base case process, GWP and FER savings of 42% and 38% were achieved when compared to fossil-derived diesel, respectively. The main bottlenecks identified include the energy requirement for algal cultivation and drying as well as embodied burdens associated with nutrient supply and construction materials. Sensitivity analysis showed that the most sustainable production process was achieved under mixotrophic cultivation having more than 75% savings in both GWP and FER when compared to fossil derived diesel.

## Acknowledgements

Victoria Adesanya acknowledges the financial support of the Gates Cambridge Trust. Erasmo Cadena is grateful to the CONACyT for the award of a postdoctoral fellowship. Dr Ottavio Croze (Department of Physics, University of Cambridge) and Dr Elena Kazamia (Department of Plant Sciences, University of Cambridge) are also acknowledged for helpful discussions.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.biortech.2014.04.051>.

## References

- Acien Fernández, F.G., Fernández Sevilla, J.M., Sánchez Pérez, J.A., Molina Grima, E., Chisti, Y., 2001. Airlift-driven external loop tubular photobioreactors for outdoor production of microalgae: assessment of design and performance. *Chem. Eng. Sci.* 56, 2721–2732.
- Adesanya, V.O., Davey, M.P., Scott, S.A., Smith, A.G., 2014. Kinetic modelling of growth and storage molecule production in microalgae under mixotrophic and autotrophic conditions. *Bioresour. Technol.* 157, 293–304.
- Benitez, J., 2009. Principles and Modern Applications of Mass Transfer Operations, 2nd ed. John Wiley and Sons, Ltd., New York.
- Bhatnagar, A., Chinnasamy, S., Singh, M., Das, K.C., 2011. Renewable biomass production by mixotrophic algae in the presence of various carbon sources and wastewaters. *Appl. Energy* 88, 3425–3431.
- Carlsson, A.S., Van Biele, J.B., Möller, R., Clayton, D., Bowles, D., 2007. Output from EPOBIO Project: Micro- and Macroalgal Utility for Industrial Application. CPL Press, York, U.K., pp. 1–86.
- Chisti, Y., 2007. Biodiesel from microalgae. *Biotechnol. Adv.* 25, 294–306.
- Clarens, A.F., Resurreccion, E.P., White, M.A., Colosi, L.M., 2010. Environmental lifecycle comparison of algae to other bioenergy feedstocks. *Environ. Sci. Technol.* 44, 1813–1819.
- Department for Transport (DfT), 2008. Carbon and Sustainability Reporting Within the Renewable Transport Obligation; Requirement and Guidance Government Recommendations to the Office of the Renewable Fuels Agency. London, U.K.
- Gallagher, E., 2008. The Gallagher review of the indirect effects of biofuels production. *Renew. Fuels Agency*, 1–92.
- GEA Process Engineering, 2009. GEA Niro Soavi, Leading Pressure. Ariete NS 3037 Brochure. GEA Niro Soavi, Prama, Italy, [www.nirosoavi.com/literature/pdfs/NS3037.pdf](http://www.nirosoavi.com/literature/pdfs/NS3037.pdf).
- Horst, I., Parker, B.M., Dennis, J.S., Howe, C.J., Scott, S.A., Smith, A.G., 2012. Treatment of *Phaeodactylum tricornutum* cells with papain facilitates lipid extraction. *J. Biotechnol.* 162, 40–49.
- Huntley, M.E., Redalje, D.G., 2007. CO<sub>2</sub> mitigation and renewable oil from photosynthetic microbes: a new appraisal. *Mitig. Adapt. Strateg. Global Change* 12, 573–608.
- International Organization for standardization (ISO), 2006a. ISO 14040, Environmental Management. Life-cycle Assessment. Principles and Framework. ISO, Geneva, Switzerland.
- International Organization for standardization (ISO), 2006b. ISO 14044, Environmental Management. Life-cycle Assessment. Requirements and Guidelines. ISO, Geneva, Switzerland.
- Khoo, H.H., Sharratt, P.N., Das, P., Balasubramanian, R.K., Naraharisetty, P.K., Shaik, S., 2011. Life cycle energy and CO<sub>2</sub> analysis of microalgae-to-biodiesel: preliminary results and comparisons. *Bioresour. Technol.* 102, 5800–5807.
- Lardon, L., Helias, A., Sialve, B., Steyer, J.P., Bernard, O., 2009. Life-cycle assessment of biodiesel production from microalgae. *Environ. Sci. Technol.* 43, 6475–6481.
- Liu, X., Clarens, A.F., Colosi, A.M., 2012. Algae biodiesel has potential despite inconclusive results to date. *Bioresour. Technol.* 104, 803–806.
- Mandil, C., Shihab-Eldin, A., 2010. Assessment of biofuels potential and limitations: a report commissioned by the IEF. *Int. Energy Forum*, 1–59.
- Mata, T.M., Martins, A.A., Caetano, N.S., 2010. Microalgae for biodiesel production and other applications: a review. *Renew. Sust. Energy Rev.* 14, 217–232.
- Molina, G.E., Belarbia, E.H., Acien F., F.G., Robles, M.A., Chisti, Y., 2003. Recovery of microalgal biomass and metabolites: process options and economics. *Biotechnol. Adv.* 20, 491–515.
- Perlack, R.D., Wright, L.L., Turhollow, A.F., Graham, R.L., Stokes, B.J., Erbach, D.C., 2005. Biomass as Feedstock for a Bioenergy and Bioproducts Industry: The Technical Feasibility of a Billion-Ton Annual Supply (DOE/GO-102005-2135). Oak Ridge National Laboratory, TN: US DOE, 78.
- Renewable Fuels Agency (RFA), 2011. Carbon and sustainability reporting within the renewable transport obligation: technical guidance part one. Renewable Fuels Agency. Version 4.2 May 2009, 1–191.
- Rodolfi, L., Zittelli, G.C., Bassi, N., Padovani, G., Biondi, N., Bonini, G., Tredici, M.R., 2009. Microalgae for oil: strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor. *Biotechnol. Bioeng.* 102, 100–112.
- Sander, K., Murthy, G.S., 2010. Life cycle analysis of algae biodiesel. *Int. J. Life Cycle Assess.* 15, 704–714.
- Schenk, P.M., Thomas-Hall, S.R., Stephens, E., Marx, U.C., Mussgnug, J.H., Posten, C., Kruse, O., Hankamer, B., 2008. Second generation biofuels: high-efficiency microalgae for biodiesel production. *Bioenergy Resource* 1, 20–43.
- Schumacher, L.G., Marshall, W., Krah, J., Wetherell, W.B., Grabowski, M.S., 2001. Biodiesel emissions data from series 60 DDC engines. *Trans. ASAE* 44, 1465–1468.
- Shahidi, F., 1991. Canola and Rapeseed: Production, Chemistry, Nutrition and Processing Technology. Van Nostrand Reinhold, New York.

- Sheehan, J., Dunahay, T., Benemann, J., Roessler, P., 1998. A Look Back at the U.S. Department of Energy's Aquatic Species Program – Biodiesel from algae. National Renewable Energy Institute, NREL/TP-580-24190, Golden, CO, p. 328.
- Sialve, B., Burnet, N., Bernard, O., 2009. Anaerobic digestion of microalgae as a necessary step to make microalgal biodiesel sustainable. *Biotechnol. Adv.* 27, 409–416.
- Stephenson, A.L., Dennis, J.S., Scott, S.A., 2008. Improving the sustainability of the production of biodiesel from rape in the UK. *Process Saf. Environ.* 86, 427–440.
- Stephenson, A.L., Kazamia, E., Dennis, J.S., Howe, C.J., Scott, S.A., Smith, A.G., 2010. Life-cycle assessment of potential algal biodiesel production in the United Kingdom: a comparison of raceways and air-lift tubular bioreactors. *Energy Fuels* 24, 4062–4077.
- Tyson, K.S., 2001. Biodiesel: handling and use guidelines. National Renewable Energy Laboratory NREL, Golden, CO.
- Weissman, J.C., Goebel, R.P., 1987. Design and Analysis of Microalgal Open Pond Systems for the Purpose of Producing Fuels; A Subcontract Report. U.S. Department of Energy, Solar Energy Research Institute, Golden, CO, pp. 1–231, Subcontract No. XK-3-03153-1.
- Wu, X., Ruan, R., Du, Z., Liu, Y., 2012. Current status and prospects of biodiesel production from microalgae. *Energies* 5, 2667–2682.
- 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. *Bioresour. Technol.* 102, 159–165.