Energy balance and environmental impact analysis of marine microalgal biomass production for biodiesel generation in a photobioreactor pilot plant

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Abstract: A life cycle assessment (LCA) and an energy balance analysis of marine microalgal biomass production were conducted to determine the environmental impacts and the critical points of production for large scale planning. The artificial lighting and temperature conditions of an indoor bubble column photobioreactor (bcPBR) were compared to the natural conditions of an equivalent outdoor system. Marine microalgae, belonging to the dinoflagellate and raphidophyte groups, were cultured and the results were compared with published LCA data obtained from green microalgae (commonly freshwater algae). Among the species tested, *Alexandrium minutum* was chosen as the target marine microalgae for biomass production under outdoor conditions, although there were no substantial differences between any of the marine microalgae studied. Under indoor culture conditions, the total energy input for *A. minutum* was 923 MJ kg^{-1} vs. 139 MJ kg^{-1} for outdoor conditions. Therefore, a greater than 85% reduction in energy requirements was achieved using natural environmental conditions, demonstrating the feasibility of outdoor culture as an alternative method of bioenergy production from marine microalgae. The growth stage was identified as the principal source of energy consumption for all microalgae tested, due to the electricity requirements of the equipment, followed by the construction material of the bcPBR. The global warming category (GWP) was 6 times lower in outdoor than in indoor conditions. Although the energy balance was negative under both conditions, this study concludes with suggestions for improvements in the outdoor system that would allow up-scaling of this biomass production technology for outdoor conditions in the Mediterranean.

Keywords: *Alexandrium minutum*, *Karlodinium veneficum*, *Heterosigma akashiwo*, pilot plant photobioreactor, life cycle assessment, energy balance.

1. INTRODUCTION

The next decade will be crucial in solving many of the environmental issues of our planet, especially those regarding the increase in greenhouse gases (GHG), water shortages, and the depletion of fossil fuels. Issues related to CO_2 emissions and fossil fuel depletion are linked, due to the large amounts of CO_2 released into the atmosphere from the industrial, transportation, and energy sectors [1]. To avoid further increases in GHG emissions and to increase the energy reserves of different countries, governments, policy stakeholders and research groups are investing in and developing projects related to the production of biofuels from terrestrial biomass feedstock, known as the "first generation" biodiesel, including corn, rapeseed, sunflowers, and sugarcane plants. There are advances in the production of "second generation" biodiesel, using residues from trees or lignocellulosic material as feedstock for bio-ethanol production. However, the use of these feedstocks for biodiesel production is controversial because the processing and commercialization of terrestrial plants are associated with several environmental and social problems, including a loss of biodiversity, increased freshwater consumption, higher prices of edible plants, and the resulting social inequalities [2]. Alternatively, one of the most promising feedstocks for the "third generation" of biodiesel production involve microalgae, due to their photosynthetic conversion efficiency, fast growth, sustainable biomass production, and high content of triacylglycerols (TAG), which is the oil that is commonly used as a raw material for biodiesel production [5],[6]. To date, freshwater microalgae have been the main microalgal species researched for biomass and biodiesel production purposes. Of particular interest are the green algae, or Chlorophycean, including *Chlorella vulgaris, Chlorella protothecoides, Chlamydomonas reinhardtii,* and *Neochloris oleoabundans,* due to their high growth rates and their well-studied life cycle [7,8]. However, a drawback to their use is the

permanent need for large quantities of freshwater in the continuous production of sufficient microalgal biomass, independent of the culture system. Use of sea/wastewater as the culture medium would significantly reduce the water footprint [9]. This implies the need to isolate seawater strains from the same place where they will later be grown. The efficient use of these strains requires that they have high TAG concentrations in addition to other energetically or commercially favorable cellular metabolites. Several advantages of the use of seawater as the medium for microalgae are that it leaves freshwater supplies free for other human and ecosystem uses, avoids ecological problems associated with the introduction of exotic microalgal species, maintains the system without any alteration to the local ecology, and avoids the loss of biodiversity [10]. The use of seawater microalgae strains allows the installation and operation of industrial scale plants in coastal countries, use non-arable land, and avoids or at least reduces freshwater consumption.

Based on these considerations, our group has explored the growth rates, lipid profiles, and TAG concentrations of various marine microalgal species and involved culturing the strains of interest in enclosed systems and improving these cultures for energetic purposes [12]. Most of the microalgae evaluated by our group in previous studies belong to the dinoflagellates and raphidophytes classes [12]. Dinoflagellates are well known because of their extensive bloom-forming proliferations in natural marine environments throughout the world [14],[15]; in terms of the production of biomass for bioenergy, this harmful trait becomes an opportunity and an advantage. Previous studies [16],[17] determined that dinoflagellates and raphidophytes readily adapt to growth in enclosed systems and that their natural capacity of proliferation can be exploited to establish long-term biomass culture facilities in various coastal countries [17,18]. The strains used in this study are present globally and can be considered strategic species

because they can be isolated readily from local seawater spots around the world [14]. *Alexandrium minutum* is a tecate dinoflagellate with a high cell biovolume (> 2800 μm^3) with a high biomass and lipid productivity. The dinoflagellate *Karlodinium veneficum* and the raphidophyte *Heterosigma akashiwo* are atecate cells and are advantagous in terms of lipid extraction by the ease of breaking the cells and avoidance of a higher energy input for the extraction of the lipids. [13].

The biotechnology used for biomass production from microalgae principally involves two types of culture configuration: open and enclosed systems. Open systems, including raceways or open ponds, have a low initial cost of construction and maintenance, with a relatively low volumetric productivity, and parameters including temperature, evaporation, and contamination cannot be totally controlled [5]. Enclosed systems, including horizontal photobioreactors, bubble columns, or flat panels, produce a higher volumetric biomass (13-fold greater than raceways or ponds), allow the growth of a single microalgal cell type (monoculture), and have fewer contamination problems than open systems. However, the initial cost of construction is higher for enclosed systems than for open systems [5]. The energy cost of microalgal biomass production in enclosed systems suffers from the current need for materials and procedures that require high amounts of energy, including the different plastics used in the construction of the photobioreactor in bubble column photobioreactors and the concrete needed for open pond systems. Electricity consumption during the microalgal growth stage (water, air pumping, CO_2 injection, etc.) or in the filtration systems used to extract the biomass from the seawater in the dewatering stage is also high. Both open and enclosed systems are used to grow microalgae under autotrophic conditions, with sunlight as the energy source, nutrients obtained from a liquid medium, and inorganic carbon, as CO_2, provided in pure form or as injected air with atmospheric CO_2 concentrations. With

these inputs, chemical energy is formed via photosynthesis [18]. Presently, most of the studies that use microalgae for biofuel purposes have been implemented in the lab or pilot scale, pending industrial scaling to demonstrate the production feasibility [7,8]. In this study, an enclosed system was chosen to achieve high marine microalgae biomass production because it allows the control of abiotic parameters and its biomass production per volumetric area is higher than in open systems. Additional considerations in establishing open system facilities are the high price of land in the Mediterranean area and the stable weather conditions in this area. The local strains of dinoflagellates and raphidophytes produce extensive natural proliferations in the Mediterranean basin [20], so these conditions were reproduced in controlled systems [12,13], together with the same abiotic parameters and seawater encountered by natural populations, following the suggestion of "built around algae" facilities for long-term microalgal biomass production [21].

Life cycle assessment (LCA) is a tool that allows the potential impacts along the life cycle of a product, process, or activity to be evaluated. LCA studies in microalgal biomass production for biodiesel purposes are principally based on models or laboratory data; however, most of the data are assumptions or refer to a hypothetical system based on extrapolations from lab-scale studies [9],[22],[23]. In this study, data for the LCA were obtained from a previous study [18], in which microalgal cultures were run in a bubble column photobioreactor (bcPBR) pilot plant under controlled conditions (indoors) and in a natural environment (outdoors). Energy balance is the key consideration in the design and development of a new methodology/feedstock aimed at energy production. Accordingly, measuring and evaluating the energy consumption of a newly proposed system simplifies improvements and facilitates increases in its efficiency.

142 The aims of the present study can be defined as follows:

143 1) To determine the energy balance of dry marine microalgal production (*A. minutum,*

144 *K. veneficum* and *H. akashiwo)* in a bcPBR pilot plant under indoor and outdoor

145 conditions.

146 2) To evaluate and determine the principal environmental and energy impacts in the

147 production of marine microalgal biomass under artificial (indoor) and natural (outdoor)

148 conditions of temperature and lighting in a bcPBR pilot plant.

149 3) To assess the relative energy and environmental contributions of LCA stages, to

150 detect the weak also in addition to the critical points of an outdoor system, with the goal

151 of obtaining a viable and scalable design for an industrial-scale biodiesel facility.

152 4) To discuss the feasibility of microalgal biomass production facilities for biodiesel

153 generation in the Mediterranean basin using outdoor conditions without the need of

154 energy inputs using artificial light and temperature control.

155 2. MATERIALS AND METHODS

156 2.1 Description of the microalgal cultivation in the pilot plant

157 The study was conducted at the Institut de Ciències del Mar (ICM-CSIC), Barcelona,

158 Spain, under ambient Mediterranean climate conditions (41º 23' 16.5" N; 02º 10' 11.71"

159 E). Three species of microalgae, two belonging to Dinophyceae (AMP4 *A. minutum* and

160 ICMB252 *K. veneficum*) and one to Raphidophyceae (ICMB830 *H. akashiwo*) were

161 grown in bubble columns under indoor and outdoor environmental conditions.

162 The experimental design consisted of a bcPBR, which has a supporting structure of

163 wood and polymethylmethacrylate tubes, as depicted in Figure 1. The

164 polymethylmethacrylate tubes (height = 2.0 m and diameter = 0.15 m) each had a

165 volume of 33 dm^3. Three tubes were used for each microalgal species, both for indoor

166 and outdoor conditions; therefore, the indoor system had a total workload of 0.297 m^3

as did the outdoor system. The bcPBR was 2.65 m in length and 0.75 m in width. The separation between the tubes was 0.11 m, with a total surface utilized of 1.98 m^2 and a volume-surface ratio of 0.15 m^3 m^{-2}. For both growth conditions, the microalgae were cultured in triplicate.

Under indoor conditions, the microalgal strains were grown in a temperature-controlled room at 20ºC ± 1ºC. All cultures were grown in filtered (0.21 µm) seawater (salinity of 37 kg m^{-3} and neutral pH) obtained from the ICM culture facilities and supplemented with a full L1-enriched medium without added silicates [24]. Pre-filtered air (Iwaki filter, 0.2 µm pore size) with a CO_2 concentration of 420 µL L^{-1} ± 16 µL L^{-1} (measured by a Qubitsystem S151 CO_2 Analyzer) was injected from the bottom of the tubes at a flow of 50c m^3 s^{-1}, which allowed gentle agitation inside the bubble column.

For outdoors conditions, a bcPBR with the same layout, seawater salinity, pH, injected air, and growth medium as used for the indoor conditions was placed on the terrace of the ICM-CSIC. The experiment started in mid November 2009 and was terminated at the end of May 2010 (autumn, winter, and spring in the northern hemisphere). Cultures were run in a semi-continuous mode because 50% of the biomass was harvested depending on the duplication time of each species (Figure 2). Throughout the experiment, light and temperature were recorded under the outdoor conditions from the Catalonia meteorological station net [25].

Figure 1. Photograph of the bubble column photobioreactor (bcPBR) under outdoor (left) and indoor (right) conditions.

To obtain dry biomass, the samples were centrifuged at 471 rad s^{-1} for 420 s in a Sigma 3-16 K centrifuge to separate the seawater from the microalgae. The supernatant water was discarded and a wet biomass pellet was recovered.

Figure 2. Growth curve for the different microalgae tested under outdoor conditions. ✦ Indicates the harvest time of the culture.

2.2 Life cycle assessment (LCA) of the microalgal biomass production in a bcPBR pilot plant

The energy and environmental assessment of the proposed experimental design was carried out using the LCA methodology. The LCA evaluates the potential impacts along the life cycle of a product, process, or activity, from raw material extraction to production, use, and disposal [26]. The ISO 14040 provides guidance on the four steps of the LCA: goal and scope, inventory analysis, life cycle impact assessment, and life cycle interpretation.

2.2.1 Functional unit and boundary system

The functional unit of this study is the production under indoor and outdoor conditions of 1 kg of dry microalgal biomass from each of the species studied. The biomass obtained would be used for biodiesel production. Figure 3 depicts the studied system and its limits. The system includes all the steps necessary to obtain dry biomass from microalgae: culture medium production, bcPBR structure production, energy consumption during the filling and dewatering stages, growth of the microalgae (indoors and outdoors), and bcPBR maintenance (cleaning). Lipid extraction and transesterification are not considered in the limits of biomass production of this LCA.

Figure 3: Life cycle system of microalgal biomass production for biodiesel production

2.2.2 Life cycle inventory

Table 1 shows the life cycle inventory and the data, which were collected and classified throughout the experiment (November 2009 - May 2010). All data are expressed per

functional unit, i.e., the production of 1 kg of dry microalgal biomass, except for the equipment, is expressed in terms of power. Table 2 details the dry biomass obtained per liter [18].

Inflows to the system included equipment power (kW), operating rates (s kg^{-1}), photobioreactor material (acrylic kg kg^{-1}), culture medium doses (kg kg^{-1}), and seawater consumption (m^3 kg^{-1}). Outflows from the system were dry biomass (kg) and the waste seawater with L1 culture medium obtained following centrifugation (kg m^{-3}). In the dewatering process, 98.5% of the water is lost as a result of the centrifugation dewatering [12]. The production inventory of the culture medium was taken from the literature and the ecoinvent database [27],[28]. Data for the electricity was obtained from the ecoinvent database as well [29].

The water and air needed for the experiment were supplied by general pumps located in the ICM which in turn supply water and air to various experiments of the research center. The total energy consumption from the water pump was calculated from the hours of working required for the experiment and pump power. The same procedure was followed for the energy consumption of the dewatering, although specific equipment was used for the experiment. Air was pumped into a tank with a flow of 202 dm^3 s^{-1} and then was provided to the experiment with a flow of 50 cm^3 s^{-1}. The total pump energy consumption was calculated considering time for tank filling and air pump power.

The total volume of the chamber used is greater than the volume required for this experiment; therefore, the total energy consumption of the chamber (28.8 m^3) was adapted to the volume of the growing tubes (0.3 m^3), taking into account the space needed between the tubes (the volume fraction is 14%). The same procedure used for the chamber was adopted to determine the energy consumption due to the fluorescent

240 lights. To calculate the bioenergy production from the biomass obtained the lipid
241 extraction and the oil transesterification should be considered. A production rate of 25%
242 lipids was measured for each microalgal species in a previous study [13,19] and a
243 transformation of 90% was considered.

244 **Table 1. Life cycle inventory of biomass production for three marine microalgal**
245 **species cultured under indoor and outdoor conditions**

246 **Table 2. Dry biomass per liter for each microalgal species and growth system**

247 2.2.2.1 Assumptions for life cycle inventory

248 In the life cycle inventory the following assumptions were made:

249 • For the bioenergy production calculation, the experimental low calorific value of
250 39 MJ kg^{-1} was used [30].

251 • The useful life of the bcPBR was estimated to be 10 years, and its total weight
252 80 kg.

253 2.2.3 Life cycle impact assessment (LCIA)

254 The SimaPro 7.1.8 software was used for the environmental evaluation together with
255 the method detailed in "CML baseline 2001." The impact categories include are: abiotic
256 depletion (AD) in kg Sb eq.; acidification (A) in kg SO_2 eq.; eutrophication (E) in kg
257 PO_4 eq.; global warming potential (GWP) in kg CO_2 eq.; ozone layer depletion (ODP)
258 in mg CFC-11 eq.; human toxicity (HT) in kg 1,4-DB eq.; freshwater aquatic
259 ecotoxicity (FWAE) in kg 1,4-DB eq.; marine aquatic ecotoxicity (MAE) in kg 1,4-DB
260 eq.; terrestrial ecotoxicity (TE) in kg 1,4-DB eq.; and photochemical oxidation (PO) in
261 kg C_2H_4 eq.

262 2.2.4 Energy assessment

263 Simapro 7.1.8 software and the "Cumulative Energy Demand v 1.4" method were used
264 in the energy assessments at all stages of the LCA. This method was used to estimate

265 the direct energy consumption, including the use of seawater and the freshwater needed
266 for the maintenance, production of culture medium and the production of bcPBR. In
267 addition, the net energy balance was determined, calculated as the difference between
268 energy output and energy input.

269 2.3 Sensitivity analysis

270 A sensitivity analysis was conducted using the variables of energy consumption and
271 lipid content of dry biomass to observe when positive balances would be achieved. The
272 analysis used results obtained for outdoor production from *A. minutum* because this
273 dinoflagellate species presented the best energy results. Five scenarios where defined as
274 A, B, C, D and E. The base case for all results reported in this LCA is calculated for the
275 algae composition of 25% lipids so the percentage of lipid content was increased at
276 intervals of 10% from the base case represented by scenario A. Energy consumption
277 was reduced at intervals of 50% from the base results obtained in the study. Both
278 variables were modified in each scenario, so in scenario B the energy consumption was
279 reduced by 50% over scenario A and lipid content increased by 10%; in scenario C
280 energy consumption was reduced by 50% over scenario B and lipid content was
281 increased again by 10%; and so on for scenarios D and E.

282 3. RESULTS

283 The following sections describe the energy balances obtained for indoor and outdoor
284 production systems and the energy and environmental assessment of the different stages
285 considered in the LCA. Finally, the data from the sensitivity analyses determined from
286 the best results (*A. minutum*) is presented.

287 3.1 Energy results

288 Table 3 lists the total energy consumption by each species of marine microalgae for
289 both production systems and the output of bioenergy production from microalgae based

290 on the inventory and the assumptions described in section 2.2.2. The energy balances
291 obtained are also presented. The results are expressed in MJ per kg of dry microalgae
292 species biomass.

293 **Table 3. Energy consumption, output and balance per kg of dry biomass for each**
294 **life cycle stage and for each microalgal species and growth system**

295 3.1.1 Energy results of production systems

296 First, it is observed from Table 3 that negative balances were obtained for both
297 productions systems. In addition, the energy balance results demonstrated large
298 differences between the indoor and outdoor systems in contrast to the biomass results
299 displayed in Table 2, in which the two systems did not differ substantially. The outdoor
300 system consumed significantly less energy than the indoor system with differences
301 between 721 and 783 MJ kg^{-1}. Specifically, *A. minutum* grown in the outdoor system
302 had the best energy balance (-139 MJ kg^{-1}) while indoor production of this same
303 microalgae had the worst balance (-923 MJ kg^{-1}).

304 3.1.2 Energy results of microalgae

305 Minor differences were found for the energy results of the different microalgal strains
306 grown in the same production system. In the case of outdoor production, energy
307 consumption differences were less than 7.5% and for indoor production the energy
308 demands differed by less than 6.0%. This means that for each type of microalgae and
309 for both systems, biomass production was robust, and in future experiments and
310 applications any microalgal species could be used.

311 3.1.3 Energy results of life cycle stages

312 The analysis of life cycle stages of both types of production and species indicated that
313 the largest contributors to the energy demand were the microalgal growth and the
314 construction of the bcPBR stages.

315 In the indoor system, the growing life stage required high energy demands for light and
316 temperature maintenance, which need to be artificially provided and controlled to
317 maintain constant environmental conditions for growth (values highlighted in gray in
318 Table 3) and using more than 85% of the electricity consumption of the entire system.
319 The elimination of these operations reduces the overall electricity consumption by 90%,
320 as observed in the outdoor system, in which temperature and light were provided
321 naturally, with no need for additional electricity input. However, the outdoor system air
322 pumping involves considerable electricity consumption in the growth stage,
323 approximately 60% of the entire system, constituting an energy demand of
324 approximately 90 MJ. Notably that the equipment used for lighting, temperature and air
325 pumping at the growth stage was adapted and not specially designed for the experiment,
326 the ecodesign of the equipment could significantly reduce the electricity consumption
327 and therefore improve the energy balance. In addition, the production of the bcPBR
328 involves a significant energy demand in both systems because the chosen material has a
329 high energy requirement in its production. The polymethylmethacrylate tubes were
330 chosen because they allow a good light penetration for photosynthesis activity and
331 prevent the aging of the material by the action of UV rays. The replacement of this
332 material by other with same characteristics or the bcPBR ecodesign could contribute to
333 reduce the energy inputs and improve the energy balances.
334 Other stages including dewatering, water consumption or L1 culture production to
335 promote microalgal growth involve lower energy consumption in both systems;
336 however, they should be considered in further research.
337 3.2 Environmental results
338 The environmental impacts of bioenergy production per functional unit were determined
339 for ten impact categories. The total environmental impact by production system and by

340 type of marine microalgae, particularly compared with the global warming category, is
341 presented followed by an evaluation of the relative contributions of the life cycle stage.
342 3.2.1 Total environmental impacts
343 For all impact categories and microalgal species, outdoor systems had lower
344 environmental impacts (see Table 4). Specifically, *A. minutum* outdoor production had
345 the lowest environmental impact in all categories (marked in black in Table 4). By
346 contrast, *A. minutum* indoor production had the highest impact (indicated in gray in
347 Table 4) for all categories. The outdoor system had significantly fewer environmental
348 impacts than the indoor systems with differences between 85% and 88%, indicating that
349 in environmental terms the outdoor system had superior results and it is therefore
350 presented as the preferable choice. Similar to energy results, there were few differences
351 between the types of microalgae, for outdoor and indoor systems the environmental
352 impacts differ less than 6% between them in all impact categories.
353 **Table 2. Environmental impacts for microalgal species and impact category**
354 Compared with the global warming (GWP) category, the indoor system production
355 yielded an average of 146.3 kg ± 4 kg of CO_2 eq. per functional unit (kg of dry
356 biomass). The outdoor production in the same category resulted in an average of 23.24
357 kg ± 0.7 kg of CO_2 eq. Thus, the GWP was 6 times lower under outdoor than indoor
358 conditions.
359 3.2.2 Environmental impacts of life cycle stage
360 To analyze in greater detail the environmental impacts by impact category, it is
361 necessary to assess the impacts by life cycle stages. Figure 4 shows the relative
362 contributions of the life cycle stages of *A. minutum* indoor production which has the
363 worst environmental impact results. The higher environmental impacts under indoor
364 conditions for *A. minutum* were due to the microalgal growth stage, which accounted for

365 more than 95% of all of the environmental impacts and is a totally function of electricity
366 consumption, i.e., temperature, light conditions requirements and air pumping. The
367 impacts are mainly due to the electricity production which depends on the Spanish
368 energy mix considered which had a contribution of 57% fossil fuel energy and 20%
369 renewable energy. The relative contribution of filling and centrifugation were less than
370 2% and were dependent on the electricity consumption and water and nutrient
371 consumption for the filling stage; thus, more than 96% of all of the environmental
372 impacts are due to electricity consumption and therefore due to the Spanish mix. A
373 change in the contributions of fossil energies would contribute to decrease the
374 environmental impacts. The remaining environmental impacts from the indoor
375 production were a consequence of the bcPBR production. A material change could
376 involve a reduction of the environmental impacts.

377 **Figure 4. Relative contributions of different life stages of *A. minutum* under indoor**
378 **conditions**

379 As was the case for the indoor production of *A. minutum*, the outdoor production of *H.*
380 *akashiwo* had the worst environmental results; therefore, its breakdown of life cycle
381 stages was chosen to analyze the environmental impacts of the outdoor system and to
382 define the principal environmental impact. The results and its relative percentages for
383 each life cycle stages are depicted in Figure 5. The electric consumption is considerably
384 lower in this system; therefore, the impacts due to other stages implied a higher relative
385 contribution for certain categories. This demonstrates that these stages are also a source
386 of impacts and should be considered.

387 **Figure 5. Relative contribution of different life cycle stages of *H. akashiwo* under**
388 **outdoor conditions.**

389 The electricity consumption yielded results of 71% (AD) and 95% (ODP-TE) in all
390 environmental impacts where the growth stage accounted for 65% (AD) and 87%
391 (ODP-TE) and the centrifuge represented approximately 7% of impacts in all categories.
392 As for the indoor system, these impacts are due to the energy mix considered. The
393 production of the bcPBR constitutes the second stage with higher impacts, and as in the
394 indoor production, the consumption of fossil fuels implies that in AD, AC, E, GWP and
395 PO, the contribution was between 14% and 24% indicating again that the reactor
396 material substitution could involve great environmental improvements.
397 The lowest environmental impacts in all of the categories were during the stage of
398 filling which depends on electricity for pumping, water and nutrients consumption.
399 Figure 6 presents their relative contributions showing that the L1 culture consumption
400 had the highest contribution in the categories of E and GWP due to the nutrient
401 consumption of nitrogen or phosphorous.
402 **Figure 6. Relative contribution of electricity, water and L1 culture consumption of**
403 ***H. akashiwo*** **under the outdoor conditions during the filling stage**
404 3.3 Sensitivity analysis
405 Sensitivity analysis of the outdoor production of *A. minutum* was performed by
406 changing the energy consumption and lipid content of the dry biomass. Table 5 displays
407 the results obtained for the scenarios defined. Positive balances were obtained for
408 scenarios D and E, which implies an energy reduction of 88% from the base results
409 presented in scenario A and a content lipid of 55%. These results demonstrate that great
410 efforts should be made to achieve positive balances of this production system. However,
411 as noted in section 3.1, there is a great potential for energy reduction if ecodesign and
412 specifically adapted equipment is used for the microalgae production and/or if the
413 bcPBR or the material itself is replaced. The environmental impacts of scenario D

414 would be reduced by 63-84%; so the emissions of CO_2 eq. would be 8.2 kg per
415 functional unit.
416 **Table 5. Sensitivity analysis after modifying energy consumption and lipid content**
417 **for scenarios A, B, C, D and E**
418 4. DISCUSSION
419 The production of microalgae in an outdoor rather than an indoor system results in a
420 slight decrease in biomass production; nevertheless, it involves a significant decrease in
421 the total energy consumption, thus outdoor systems are presented as a preferable option.
422 This study was conducted on experimental data from a pilot plant and a key aspect was
423 that the equipment used was not specifically designed for the experiment. However, this
424 is the first step to properly scale an experiment and the joint analysis of production,
425 energy and environmental impacts allows us to establish what the weakest points are on
426 which further research or greater effort must be applied. The results of the pilot plant
427 production indicate that outdoor production is possible and that the differences are
428 notably small with controlled productions. However, future studies should take into
429 account that biomass productivities in outdoor photobioreactors naturally illuminated
430 would depend on the prevailing weather conditions in a particular locality [31]. Under
431 Mediterranean climate conditions, our outdoor production system yielded similar or
432 superior results as obtained for green algae in others studies based on the same
433 geographical area [32,[33], and the differences between the marine microalgal species
434 studied in this study were so small that the production of any of them would be possible.
435 In recent years, many LCA and energy balance studies on the microalgae production for
436 energetic purposes have been conducted [34-43]; however, there is an enormous variety
437 of microalgae species that can be used to produce biodiesel and many different methods
438 of microalgal cultivation. In addition, the life cycle stages included in each study may

vary, thus, while certain studies have analyzed the entire cycle [34],[41]] others have only considered the culture process [38]. The results of several of these studies are presented in Table 6. However, due to methodological and life cycle differences, general comparisons and extrapolations are difficult.

Table 6. Schemes of various LCA studies of bioenergy from microalgae

The energy assessment indicates negative balances for both indoor and outdoor production systems; however, for the latter, positive balances can be gained by reducing energy consumption. In addition, for all the studies complied in Table 5 [37]-[40], negative balances are obtained except for [38] when raceway pond and flat-plate PBR are considered. These types of reactors consume considerably less energy than tubular PBRs [44],[45] or open ponds [40], thus an alternative strategy to decrease energy consumption would be to use an outdoor system based on a raceway pond inside a greenhouse. Nonetheless, in places in which evaporation is high, raceway ponds require more frequent water pumping than tubular bioreactors [41], which would increase energy consumption, and this needs to be taken into consideration**.** In addition, raceway or open ponds should be implemented in those countries with extensive non-arable or inexpensive land (e.g., North African countries). In contrast, in those countries in which high land prices limit the system (EU Mediterranean countries), bcPBRs or other enclosed systems is a reasonable choice. In addition, the production of bcPBR has been observed to be the second highest source of energy consumption due to material election. As indicated by [40], one of the disadvantages of such reactors is that their construction requires sophisticated materials. Thus, innovations and ecodesign in the layout and construction materials would significantly reduce the energy consumption associated with its production and decrease the overall energy requirements. These innovations include the combination of advanced designs of synthetic bags floating

464 partially submerged in an artificial pond (a combination of open and enclosed systems),
465 or a single reactor module consisting of one large translucent plastic bag containing
466 multiple vertical panels [21].
467 Downstream processing, i.e., dewatering and lipid extraction, have been observed as
468 important stages and should be considered in energy balances [46],[47]. In a previous
469 study [39], dewatering constitutes the largest energy input, consuming 54 MJ per kg of
470 dry biomass due to natural gas consumption. However, a different study [40] carried out
471 a comparative LCA on dry and wet dewatering, and the dry process consumed 4.7 MJ
472 per kg of dry biomass due to a centrifuge (similar to our study) in which energy
473 consumption resulting from dewatering is 6 and 8 MJ kg^{-1} for outdoor and indoor
474 systems, respectively. The lipid extraction is not discussed; however, certain authors
475 found the highest energy consumption as a result of this stage [42],[43]. Further studies
476 must be conducted to establish the best options for the dewatering alternatives and lipid
477 extraction processes.
478 The use of a culture medium to promote microalgal growth is the life cycle stage with
479 the lowest energy consumption, which contrasts with results found in a previous study
480 [37] and with terrestrial crops for biofuel purposes, in which energy consumption
481 related to crop fertilization and to production could be the highest in the entire cycle.
482 Fertilizer manufacture itself amounts to 46% in the establishment of the crop and 32%
483 in the first cycle [48] for a LCA conducted of a *Populus spp*. crop.
484 Relative to environmental impacts, the use of microalgae production has been promoted
485 in part as a means to reduce CO_2 emissions and improve sustainability [49],[50]. Certain
486 previously reported LCA studies have also conducted environmental analyses [39],[41].
487 The environmental results of our study demonstrated that main environmental impacts
488 are due to electricity consumption and for the global warming category (GWP) the

489 emission of 0.16 kg CO_2 eq. per MJ were found. Lower results of 0.07 kg and 0.06 kg
490 per MJ were reported by other studies [39,41]. However, results from the sensitivity
491 analysis demonstrate that positive balances could be achieved by reducing the GWP to
492 0.06 kg MJ^{-1}.
493 Finally, there is a need to standardize data quality for the inventory used, especially for
494 the purpose of comparing studies. Our study used experimental data, whereas in most
495 cases, the data were obtained from a bibliographic inventory or were extrapolated from
496 industrial processes used for other modes of generic biofuel production. In this sense,
497 the energy balances obtained may not be consistent.

498 5. CONCLUSIONS

499 In Mediterranean outdoor conditions, marine microalgae production for biodiesel is a
500 good option and a feasible route to obtain bioenergy. We recommend that production
501 and research under indoor conditions be rejected based on the energy results obtained.
502 However, for outdoor systems, efforts should be made to decrease energy consumption.
503 As revealed herein, the highest energy consumption occurs during the growing stage
504 due to the mechanical requirements of the pumps and the need for air injection. Thus,
505 for industrial scale improvements, more efficient equipment is needed. In the same
506 manner, more energy-conserving bcPBR material or its eco-design could significantly
507 reduce energy consumption. Any of the three microalgae analyzed can be cultivated and
508 exploited on a large scale as there were no substantial differences in biomass production
509 between them. In addition, the use of any of these marine microalgae leaves freshwater
510 for other human uses and thus helps to overcome the critical issue of freshwater
511 consumption in the production of microalgae. This would improve the feasibility of

512 bioenergy in terms of its large scale production and the scarcity of freshwater in the
513 Mediterranean area.
514 Other experiments should be conducted to assess productivities in Mediterranean
515 climates for spring-summer periods to evaluate whether higher productivities are
516 achieved and less energy is needed. Besides biodiesel production, additional research is
517 needed to identify the coproducts for bioenergy and other purposes.

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528

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Table

Table 1: Life cycle inventory of biomass production per functional unit for three marine microalgal species cultured under indoor and outdoor conditions

	INPUT																		OUTPUT	
	Struct	Filling						Growing of microalgae						Dewatering		Maintenance			Prod.	WSW
	bcPBR	Water pump		SW	Nutrient L1			Chamber		Air pump		Fluorescence		Centrifuge		Washing			Bio	WSW
	kg	**kW**	**s**	**m^3**	**A(kg)**	**B(kg)**	**C(kg)**	**kW**	**s**	**kW**	**s**	**kW**	**s**	**kW**	**s**	**m^3**	**kW**	**s**	**kg**	**m^3**
***H.A.* I**	0.2	0.01	4.4E+04	0.8	4.3E-03	2.8E-03	1.0E-06	0.5	1.2E06	0.02	2.4E6	0.13	1.2E06	0.46	1.3E4	0.05	0.42	6.7E3	1.0	0.8
***H.A* O**	0.3	0.01	5.6E+04	1.0	4.6 E-03	3.6 E-03	1.0E-06	0.0	0.0	0.02	3.1E6	0.0	0.0	0.46	1.8E4	0.06	0.42	8.7E3	1.0	1.0
***A.M.* I**	0.2	0.01	4.6E+04	0.8	5.6 E-03	3.6 E-03	1.0E-06	0.5	1.3E6	0.02	2.6E6	0.13	1.3E6	0.46	1.4E4	0.05	0.42	7.1E3	1.00	0.8
***A.M.* O**	0.3	0.01	5.3E+04	1.0	5.2 E-03	3.4 E-03	1.0E-06	0.0	0.0	0.02	3.0E6	0.0	0.0	0.46	1.6E4	0.06	0.42	8.1E3	1.00	0.9
***K.V.* I**	0.2	0.01	4.5E+04	0.8	4.5 E-03	2.9 E-03	1.0E-06	0.5	1.3E6	0.02	2.5E6	0.13	1.3E6	0.46	1.4E4	0.05	0.42	7.0E3	1.00	0.8
***K.V.* O**	0.3	0.02	5.6E+04	1.0	5.5 E-03	3.5 E-03	1.0E-06	0.5	0.0	0.02	3.1E6	0.0	0.0	0.46	1.7E4	0.05	0.42	8.6E3	1.00	1.00

A: fertilizers N/P/K, B: metals, C: vitamins

Table 2. Dry biomass per liter for each microalgal specie and growth system

Heterosigma akashiwo **(gL^{-1})**		***Alexandrium minutum*** **(gL^{-1})**		***Karlodinium Veneficum*** **(gL^{-1})**	
Indoor	Outdoor	Indoor	Outdoor	Indoor	Outdoor
1.25	0.97	1.18	1.03	1.2	0.98

Table 3. Energy consumption, output and balance per kg of dry biomass for each life cycle stage and for each microalgal species and growth system

		Heterosigma akashiwo		*Alexandrium minutum*		*Karlodinium veneficum*	
		Indoor	Outdoor	Indoor	Outdoor	Indoor	Outdoor
Input ($MJkg^{-1}$)	**bcPBR**	30.60	39.60	32.15	36.50	32.15	37.98
	Filling and culture						
	Filling (water pump)	0.13	0.17	0.13	0.16	0.13	0.17
	Filling (seawater)	0.24	0.31	0.26	0.29	0.25	0.31
	Culture	0.26	0.30	0.34	0.32	0.27	0.34
	Growing of microalgae						
	Chamber	598.37	0.00	633.87	0.00	623.30	0.00
	Air pump	73.47	94.98	77.83	89.17	76.54	93.72
	Fluorescents	158.09	0.00	167.47	0.00	164.68	0.00
	Dewatering						
	Centrifuge	6.21	8.00	6.57	7.53	6.46	7.92
	Maintenance						
	Washing pump	2.80	3.61	2.97	3.40	2.92	3.57
	Water	0.31	0.40	0.32	0.37	0.32	0.39
	Total	**872**	**148**	**923**	**139**	**908**	**146**
Output ($MJkg^{-1}$)		**8.78**	**8.78**	**8.78**	**8.78**	**8.78**	**8.78**
Balance ($MJkg^{-1}$)		**-863**	**-139**	**-914**	**-130**	**-899**	**-137**

Table 2. **Environmental impacts for microalgal species and impact category. Abiotic depletion (AD); acidification (A), eutrophication (E), global warming potential (GWP); ozone layer depletion (ODP); human toxicity (HT); freshwater aquatic ecotoxicity (FWAE); marine aquatic ecotoxicity (MAE); terrestrial ecotoxicity (TE) and photochemical oxidation (PO)**

Impact category (Eq. Units)	*Heterosigma akashiwo*		*Alexandrium minutum*		*Karlodinium veneficum*	
	Indoors	Outdoors	Indoors	Outdoors	Indoors	Outdoors
A.D (kg SB eq.)	1.06E+00	1.75E-01	1.12E+00	1.69E-01	1.10E+00	1.73E-01
A.C (kg SO_2 eq.)	1.36E-00	2.01E-01	1.44E+00	1.94E-01	1.42E+00	1.99E-01
E (kg PO_4 eq.)	7.02E-02	1.14E-02	7.45E-02	1.09E-02	7.32E-02	1.13E-02
GWP (kg CO2 eq.)	1.44E+02	2.38E+01	1.53E+02	2.29E+01	1.51E+02	2.35E+01
ODP (kg CFC-11eq.)	7.59E-06	9.82E-07	8.66E-06	1.63E-06	7.99E-06	9.72E-07
HT (kg 1,4-DB eq.)	4.29E+01	5.82E+00	4.56E+01	5.64E+00	4.47E+01	5.77E+00
FWAE (kg 1,4-DB eq.)	9.57E+00	1.35E+00	1.02E+01	1.30E+00	9.97E+00	1.33E+00
MAE (kg 1,4-DB eq.)	2.42E+04	3.19E+03	2.57E+04	3.11E+03	2.52E+04	3.16E+03
TE (kg 1,4-DB eq.)	2.41E-00	3.10E-01	2.56E+00	3.04E-01	2.51E+00	3.07E-01
PO (kg C_2H_4 eq.)	5.05E-02	7.74E-03	5.37E-02	7.47E-03	5.27E-02	7.65E-03

Table 5: Sensitivity analysis after modifying energy consumption and lipid content for scenarios A, B, C, D and E

	MJ kg^{-1} input	MJ kg^{-1} output	MJ kg^{-1} Balance
Scenario A	139	9	-130
Scenario B	69	12	-57
Scenario C	35	16	-19
Scenario D	17	19	2
Scenario E	9	23	14

Table 6: Schemes of various LCA studies of bioenergy from microalgae

Author	Microalgae	Reactor	E. consumption ($MJkg^{-1}$)			Balance
			Reactor	Growing	Dewatering	
Razon et al. (2011)[37]	*Haematococcus pluvialis* ***(freshwater)***	PBR +raceway pond	-	83.1	17	-134
	Nannochloropsis sp ***(seawater)***	Raceway pond	-	151	-	-465
Jorquera et al. (2010)[38]	*Nannochloropsis sp* ***(seawater)***	Raceway pond	4.5a	3.8b	-	23.3(a+b)/27.7b
	Nannochloropsis sp ***(seawater)***	Flat-plate PBR	7.3a	7.0b	-	17.3(a+b)/24.6b
	Nannochloropsis sp ***(seawater)***	Tubular PBR	-	159.0b	-	-127b
Sander et al. (2010)[39]	-	PBR and raceway pond	-	0.1	53.9	-49
Xu et al. (2011)[40]	*Chlorella vulgaris* ***(freshwater)***	Open pond dry route	0.8	3.3	4.7	-5.2
		Open pond wet route	1.0	2.2	0.40	-5.8
This work	*Alenxandrium minutum* ***(seawater)***	bcPBR	36.5	89.17	7.53	-130

[a]Energy required for reactors production
[b]Only included the energy consumption required for air pumping

Figure 1. Photograph of the bubble column photobioreactor (bcPBR) under outdoor (left) and indoor (right) conditions.

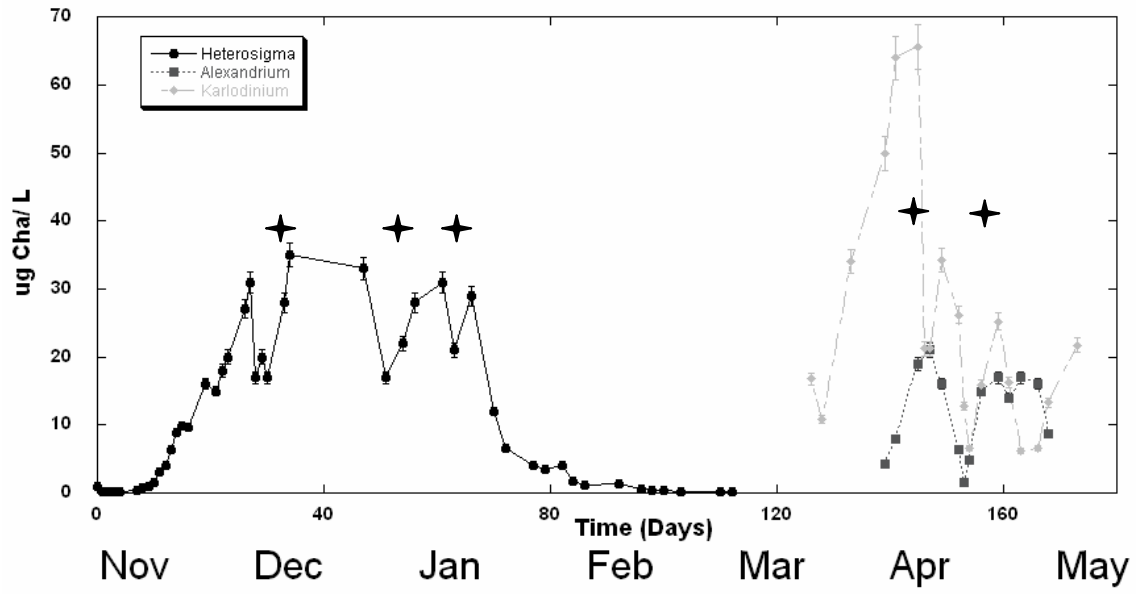

Figure 2: Growth curve of the different microalgae tested under outdoor conditions. ✦ Indicates the harvest time of the culture.

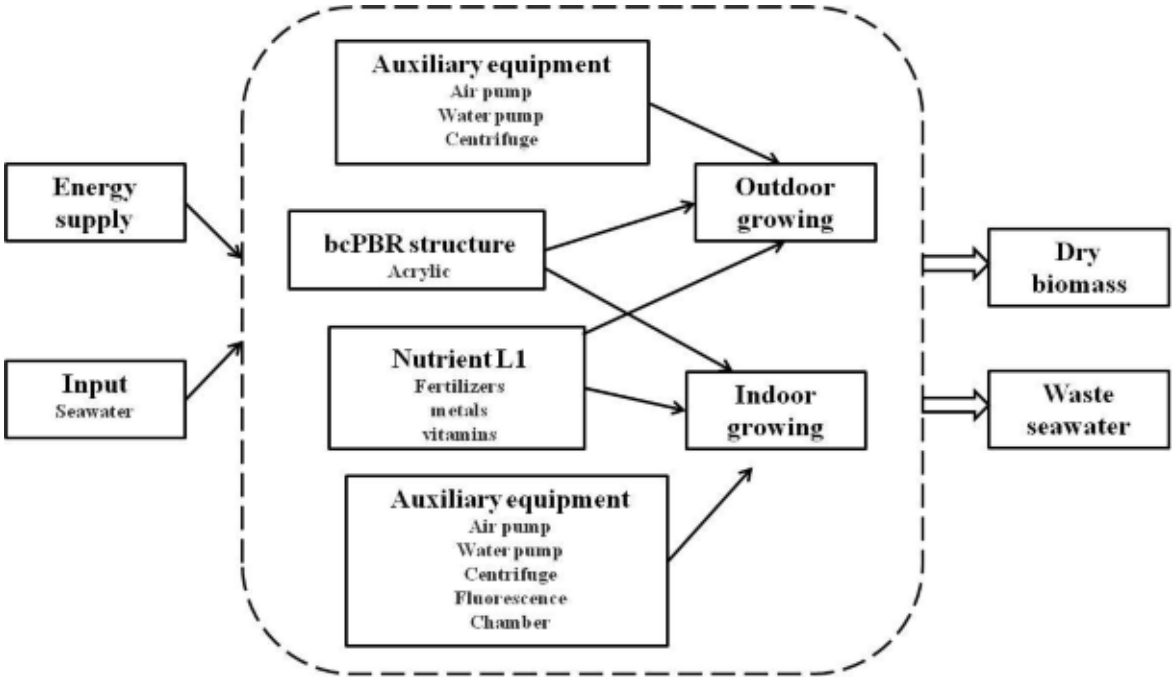

Figure 3: Life cycle system of microalgal biomass production for biodiesel production

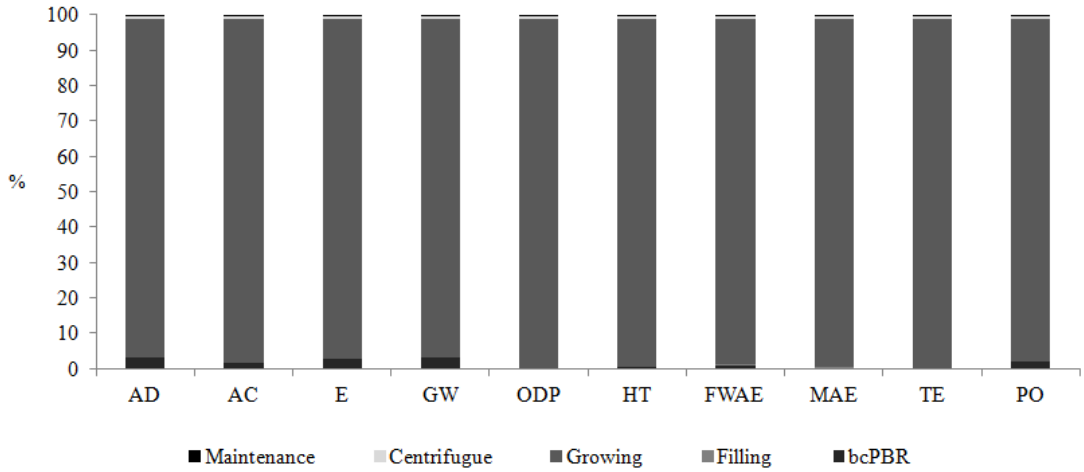

Figure 4: Relative contributions of different life stages of *A. minutum* under indoor conditions.

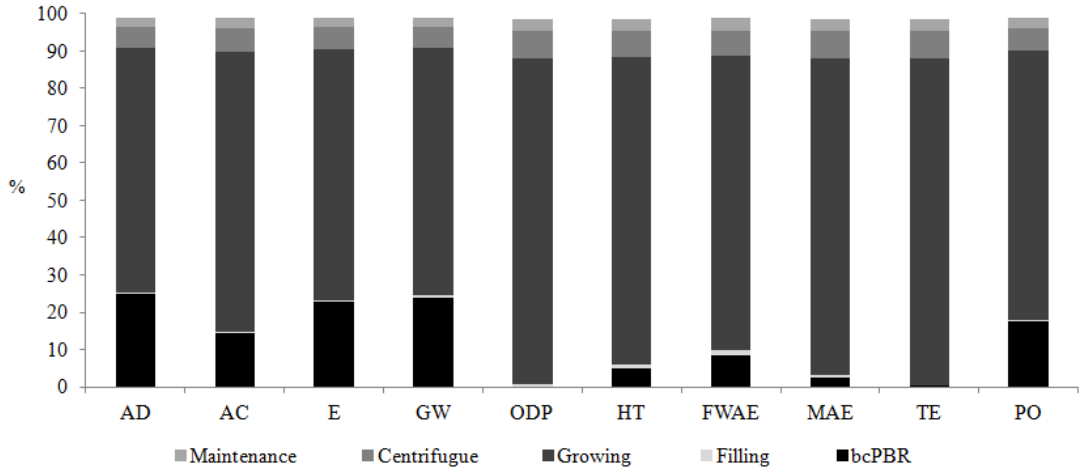

Figure 5: Relative contribution of different life cycle stages of *H. akashiwo* under outdoor conditions.

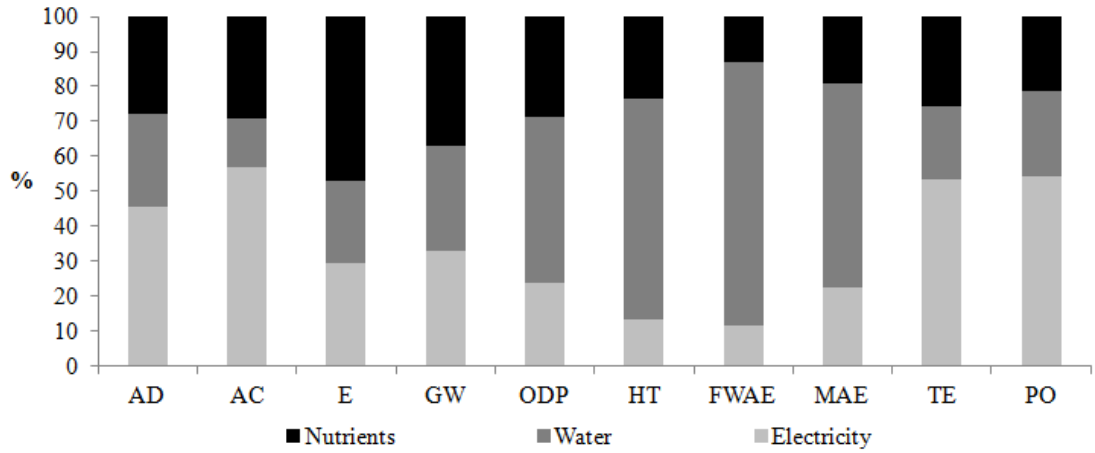

Figure 6. Relative contribution of electricity, water and L1 culture consumption of *H. akashiwo* under the outdoor conditions during the filling stage