Sustainability Evaluation of Biodiesel Produced from Microalgae *Chlamydomonas* sp Grown in Brewery Wastewater

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This study performs a sustainability evaluation of biodiesel from microalga *Chlamydomonas* sp. grown in 20 % (v/v) of brewery's wastewater, blended with pentose sugars (xylose, arabinose or ribose resulting from the hydrolysis of brewer's spent grains (BSG). The life cycle steps considered for the study are: microalgae cultivation, biomass processing and lipids extraction at the brewery site, and its conversion to biodiesel at a dedicated external biofuel's plant. Three sustainability indicators (LCEE, FER and GW) were considered and calculated using experimental data. Literature data was used, whenever necessary, to complement life cycle data, thus allowing a more accurate sustainability evaluation. A comparative analysis of the biodiesel life cycle steps was also conducted, with the main goal of identifying which steps need to be improved. Results show that biomass processing, especially cell harvesting, microalgae cultivation, and lipids extraction are the main process bottlenecks. It is also analysed the influence on the microalgae biodiesel sustainability of adding each pentose sugar to the cultivation media, concluding that it strongly influences the biomass and lipid productivity. In particular, the addition of xylose is preferable in terms of lipid productivity, but from a sustainability point of view, ribose is the best, though the difference from xylose is not significant. Nevertheless, culture without pentose addition presents the best sustainability results.

1. Introduction

Nowadays fossil fuels are cheaper and readily available, but one needs to find alternatives to them, as they are non-renewable and their real costs are on their long-term impacts on the environment and on sustainable development (Caetano et al., 2013a). Of the many options available, microalgae are seen as one of the best potential feedstocks for producing sustainable fuels for transportation (Mata et al., 2013a). However, the environmental impacts caused by freshwater consumption, nutrients supply, carbon dioxide handling, among others, can lead to constraints when designing and implementing microalgae cultivation and production systems (Chisti, 2013). Trying to make these processes economically viable and more environmentally sound, special efforts should be put on using cheap sources of carbon and nutrients or inexpensive culture systems (Mata et al., 2010). Also, microalgae cultures in mixotrophic growth conditions can potentially result in higher biomass productivities, and may become economically viable by using low- cost carbon sources such as industrial by-products, or even residual streams containing nutrients such as nitrogen and carbon (Mata et al., 2012).

Hence, this study evaluates the sustainability of biodiesel produced from microalga *Chlamydomonas* sp. cultivated in brewery's wastewater, blended with pentose sugars resulting from the hydrolysis of BSG (a byproduct of the brewery industry), which experiments are also performed in this study. Besides lowering the raw-material costs, one expects this will reduce the freshwater consumption, and thus, the environmental impacts. The addition of pentose sugars aims to balance the C/N ratio (carbon/nitrogen) of carbon poor wastewaters, while using difficult to ferment sugars resulting from BSG valorisation (Caetano et al., 2013b), as pentose sugars can only be fermented by particular yeasts.

2. Materials and Methods

2.1 Experimental study of microalgae cultivation

A culture of *Chlamydomonas* sp. (SAG 9.98) obtained from the German SAG (Sammlung von Algenkulturen Göttingen) collection was used in this study.

To start the cells acclimatization, test tubes were inoculated with *Chlamydomonas* sp. in approximately 10 mL of Bold Basal Medium with 3-fold Nitrogen (BBM-3N), that has on its composition the triple of the N amount of the original formulation (Nichols and Bold., 1965), and closed with cotton plugs (allowing air diffusion). About 25 mL of the dense test tube cultures (with 25 days from the inoculum) were transferred to 250 mL Erlenmeyer flasks and supplemented with fresh culture medium. Then, 150 mL of these cultures were transferred, after more 25 days, from the 250 mL Erlenmeyer flasks to 1,000 mL Erlenmeyer flasks and supplemented with fresh culture medium. Finally, mother cultures were prepared using about 750 mL of these pure dense cultures, after 25 days more, and supplemented to 5,000 mL with fresh culture medium. These were allowed to grow until reaching dense cultures at stationary phase, i.e. up to stabilization of the absorbance value. The BBM-3N was used until this stage so that microalgae could achieve a dense culture in the shortest time possible, by supplying all the necessary nutrients for growth, before starting the experiments using real brewery wastewater blended with pentose sugars.

The algae growth was followed in time while keeping constant the culture conditions such as room temperature (22 ºC ± 3 ºC), light/dark (L/D) photoperiod (12/12 h), and light intensity (3,000 Lux) provided by fluorescent lamps (36 W, Sylvania Aquastar T8) and measured with a luxmeter (Lutron LX-1102). Air sterilized by filtration (with a Chromafil glass fibre (GF) /polyester (PET) filter with 0.2 µm pore diameter) was supplied to the microalgae cultures at an average flowrate of 2 mL/s (for 250 mL Erlenmeyer flasks) and 4 mL/s (for 1,000 and 5,000 mL flasks) by air pumps (Pacific AP6), through the perforated rubber stopper of the flasks.

The wastewater was collected in the treatment plant of a local Brewery, after the anaerobic digestion treatment. At the laboratory, the brewery wastewater was filtered in an ultrafiltration system with 5 µm pore membranes and passed through a UV sterilization module, at a flow rate of about 70 L/ h, and again, vacuum filtered with glass fibre membranes (47 mm x 1.6 µm) to remove any remaining particles. Its analysis revealed a COD of 740.8 mg O_2/L, total phosphorus of 53.9 mg P/L, and nitrates of 17.3 mg NO_3/L.

For the study of the microalgae growth in brewery wastewater, blended with pentose sugars, about 200 mL of brewery wastewater (20 %, v/v), 700 mL of distilled water and 100 mL of the *Chlamydomonas sp* mother culture was added, to make up 1 L total volume. The wastewater dilution was necessary to attenuate its dark colour and turbidity. Then, depending on the pentose sugar test, xylose, ribose or arabinose (Merck, p.a. grade) was also added in two moments, at the inoculum stage (day 0), and during the microalgae stationary growth phase (between day 15 and 20), in concentrations of respectively, 0.25 and 0.5 g of sugar/ L of culture. It was not evaluated the use of directly brewery-derived pentoses instead of the analytical grade sugars, in order to be possible to evaluate the influence of each sugar used separately, and as it is easier to replicate the study when using analytical grade reagents instead of the ones derived from the hydrolysis of the brewer' spent grains.

The evaluation of the microalgal growth was performed in a UV/Vis spectrophotometer (Shimadzu UV- 160A), by the optical density method, and the calibration curve of absorbance vs concentration of dry biomass was obtained at 682.5 nm. All assays were performed in triplicate, so as the readings and measurements of control values, which were done regularly from day 0 until the end of the test. Thus, the results data presented are average values.

At laboratory, microalgae were harvested by centrifugation and lipids were extracted using the Bligh and Dyer method (1959).

2.2 Sustainability evaluation methodology, assumptions and indicators

The methodology used for the sustainability evaluation is described by Mata et al. (2013b), in which the full fuel life cycle (Mata et al., 2005) is taken into account, including the following steps: microalgae cultivation, harvesting, biomass processing for lipids extraction, microalgae oil transportation, biodiesel production and its final use. The biomass processing involves three sub-steps: dewatering, high pressure homogenization and three phase centrifugation.

For microalgae cultivation, open ponds were considered since they are easier and cheaper to operate than other large scale cultivation systems, in particular photobioreactors, even though less efficient from a biomass productivity perspective (Mata et al., 2010). For microalgae harvesting in this study it was considered a disk stack centrifuge. Mechanical cell disruption in a high pressure homogenizer was considered in this study, having the advantages of no chemicals needed and preservation of the cell components. After this operation, lipids were extracted and separated in a three-phase continuous centrifuge, following the suggestion of Benemann and Oswald (1996) of mixing the biomass at the entrance with a fraction of the lipids obtained in the centrifuge to increase the overall separation efficiency, up to 98 %. For biodiesel production it was considered the transesterification of triglycerides in stoichiometric excess of methanol, homogenous catalyzed by a strong alkali-catalyst (usually NaOH) (Khoo et al., 2011). Due to equipment constraints, for harvesting

and biomass processing it was considered a semi continuous operation mode, of 8 hours cycles. The cultivation and biomass processing occurred at the brewery site and then, the extracted oil was transported to a dedicated external biofuel's plant at about 50 km distance from the brewery.

The functional unit of 1 MJ of energy output from the final biofuel is considered in this study.

Three sustainability indicators were selected for this study: the life cycle energy efficiency (LCEE), Fossil Energy Ratio (FER) and Contribution to Global Warming (GW) calculated as Eq (1), (2) and (3), respectively. LCEE is the ratio of the total energy output, consisting of the energy content of the biofuel, plus that of byproducts only if they are used to supply energy to the biofuel production system, to the amount of energy expended to obtain the biofuel (dimensionless).

$$Life\ Cycle\ Energy\ Efficiency (LCEE) = \frac{Total\ energy\ Output}{Total\ energy\ Input} \tag{1}$$

FER is the ratio between the energy content of the final fuel product (or the fuel energy output) and the amount of fossil energy input (or the non-renewable energy) required for the fuel production through the supply chain

$$Fossil\ Energy\ Ratio\ (FER) = \frac{Fuel\ \ Energy\ \ Output}{Fossil\ Energy\ \ Input} \tag{2}$$

GW measures the potential contribution of different GHG (greenhouse gas) emissions to global warming, expressed as equivalent CO_2 emission per unit energy of fuel product (kg CO_2-eq/MJ fuel).

$$Contribution\ to\ Global\ Warming\ (GW) = \sum_i GWP_i \times E_i \tag{3}$$

where E_i is the mass of compound i emitted to the air and GWP_i is the global warming potential of the compound i, calculated as the net GHG emissions through the fuel life cycle.

The energy consumption data to operate each process unit was taken from literature. For example the electricity for operating the disk stack centrifuge (Molina Grima et al., 2003) or the energy for biodiesel production (Khoo et al., 2011) as well as the fossil fuel needs for the microalgae oil transportation (Mata et al., 2011). The net GHG emissions from each process technology were calculated based on the energy consumption data, either for the process units operation (ERSE, 2012) or the biodiesel tailpipe emissions (SunEarthTools, 2012). This considers the energy mix typical of Portugal and that to generate the electrical energy required to power the process units, 0.094 kg of CO_2-equivalent is emitted per MJ of electricity (ERSE, 2012). Thus, all the life cycle steps' GHG emissions are summed and the carbon capture during the microalgae growth is subtracted. For estimating this last value it was considered that the algal biomass has a molecular composition of $C_{106}H_{263}O_{110}N_{16}P$ (Shurin et al., 2013). Hence, as a consequence of microalgae photosynthesis nitrate and phosphate are taken up together with carbon in the mass proportion of C/N/P of about 106:16:1.

The utilization of the algal cake after the lipids extraction or the treatment of the biomass water after the microalgae harvesting were not considered in this analysis, as well as other emissions from the operation of process units. Thus, the main calculations involved in the sustainability evaluation are: the energy consumption of the process units that can be obtained from their technical descriptions, and the GHG emissions.

3. Results and Discussion

3.1 Microalgae growth in brewery wastewater blended with pentose sugars

Table 1 summarizes the results obtained, in terms of microalgae lipid content, biomass concentration, areal biomass productivity, lipid productivity, residence time in photobioreactor, and estimated life cycle energy needs for biodiesel production, of microalgae *Chlamydomonas sp* grown in brewery's wastewater, 20 % (v/v) diluted in freshwater, and blended with pentose sugars: xylose (CsXil), ribose (CsRib) and arabinose (CsAra) in comparison with no sugars addition (CsAq).

Table 1: Summary of results obtained for microalgae Chlamydomonas sp. *grown in 20 % (v/v) brewery's wastewater diluted in freshwater without sugars addition (CsAq) and blended with pentose sugars: xylose (CsXil), ribose (CsRib) and arabinose (CsAra)*

Growth conditions	Microalgae lipid content (% dwt)[a]	Biomass concentration (kg/m^3)[a]	Areal biomass productivity (g/m^2.d)[a]	Microalgae residence time (d)[a]	Lipid productivity (mg/L.d)[a]	Life cycle energy needs (MJ /MJ biodiesel)[b]
CsXil	25.5%	0.229	5.72	10.0	4.86	2.33
CsRib	26.0%	0.253	4.00	12.0	3.47	2.22
CsAra	39.0%	0.122	1.93	15.0	2.51	2.78
CsAq	53.5%	0.176	3.10	24.0	5.53	1.48

(a)   Determined experimentally; (b) Estimated based on the assumptions described in session 2.2

As described in session 2.1, the initial acclimatization of microalgae cells was done in BBM-3N, having 547 mg/L nitrate (NO_3). After reaching a dense culture in this medium, started the trials in real brewery wastewater (20 %, v/v) diluted with distilled water. Since the brewery wastewater has just 17.3 mg NO_3/L of nitrates, it was induced a nutrients stress caused by nitrogen deprivation. This nutrients stress contributed to the accumulation of 53.5 wt% lipids in the microalgae cells, which is much above the average values (10 -30 wt% lipids) found in literature for several microalgae species (Mata et al., 2010). This is shown in Table 1 for the microalgae grown in brewery wastewater without the pentose sugars addition (CsAq). For the cases where pentose sugars were added, the lipids accumulation was not so significant (25 – 39 wt%), which in part is due to the faster biomass growth, reaching higher biomass concentration is shorter periods of time.

Results show that in terms of lipid productivity and estimated life cycle energy needs for biodiesel production, the microalgae growth in brewery wastewater blended with pentose sugars offers no advantage in comparison with growth without sugars addition. Although without sugars addition the microalgae residence time is not the shortest, the microalgae lipid content and productivity are the highest, and the estimated life cycle energy needs are the lowest.

Comparing the effect of pentoses, xylose promoted the highest lipid productivity in the shortest growth time, but the estimated life cycle energy needs are slightly lower for ribose blended culture medium, which has a slightly higher biomass concentration that facilitates harvesting.

3.2 Microalgae biodiesel sustainability evaluation

Figure 1 shows the three sustainability indicators (LCEE, FER and GW) and Figures 2a) and 2b) the relative percentage of each life cycle step contributing respectively, to the energy consumption and net GHG emissions of producing biodiesel from microalgae *Chlamydomonas* sp. grown in 20 % (v/v) brewery's wastewater blended with pentose sugars: xylose (CsXil), ribose (CsRib) and arabinose (CsAra) in comparison with no sugars addition (CsAq).

Results show that in terms of sustainability, the microalgae growth in brewery wastewater blended with pentose sugars offers no advantage in comparison with the growth without sugars addition.

Comparing the effect of pentose sugars, for ribose the best LCEE and FER (the highest) and GW (the lowest) was obtained, since the life cycle energy needs are the lowest (as shown in Table 1). However, this is not significantly different from xylose that, among pentoses, presented the best results in terms of lipid productivity.

The small difference observed among the sustainability indicators of xylose and ribose blended medium is mainly attributed to the biomass harvesting step of their life cycles (as shown in Figure 2a). The biomass concentration obtained for ribose is slightly higher than for xylose blended medium, needing slightly less energy for biomass harvesting, which is the most significant life cycle step in terms of energy consumption (Figure 2a) and thus, of GW (Figure 2b). However, since the lipid productivity of microalgae grown in xylose blended medium is higher than that for ribose, the energy needs for lipid extraction (in the 3-phase centrifugation) is lower (as shown in Figure 2a) and thus, smaller emissions or GW (as shown in Figure 2b).
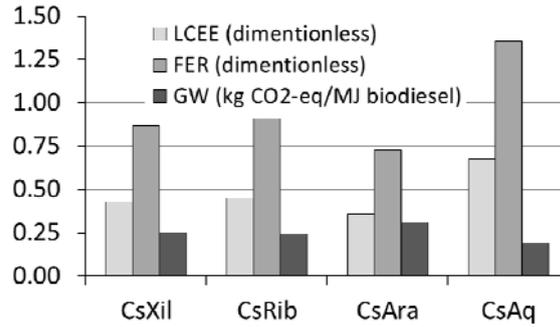
Figure 1: Sustainability indicators LCEE, FER and GW for biodiesel production from microalgae Chlamydomonas sp. grown in 20 % (v/v) brewery's wastewater without sugars addition (CsAq) and blended with pentose sugars: xylose (CsXil), ribose (CsRib) and arabinose (CsAra).
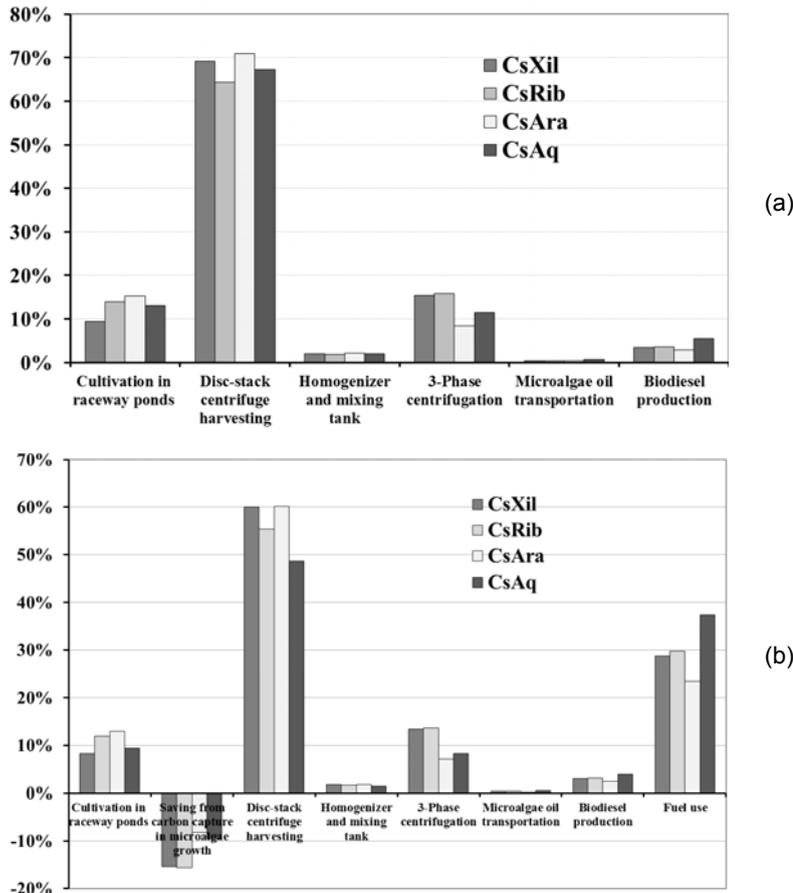

Figure 2: Relative percentage of each life cycle step contribution to the (a) energy consumption and to the (b) net greenhouse gas emissions, of producing biodiesel from microalgae Chlamydomonas sp. grown in 20 % (v/v) brewery's wastewater without sugars addition (CsAq) and blended with pentose sugars: xylose (CsXil), ribose (CsRib) and arabinose (CsAra)

Figure 2b shows that, when considering the overall life cycle, harvesting followed by fuel use, are the most relevant steps in terms of GHG emissions. Cultivation and biomass processing for lipids extraction are also relevant to a lesser extent as expected, since these have also higher relative energy consumption (Figure 2a). The savings from carbon capture during microalgae growth is also relevant but this value is indicated as negative since it needs to be subtracted from the overall life cycle GHG emissions in order to determine the net GHG emissions and thus, the contribution to global warming. As expected, at higher areal biomass productivity the relative saving from carbon capture is higher and the relative contribution to GHG of fuel use is smaller. This is expected because smaller quantities of algal biomass need to be cultivated and processed to obtain the same amount of energy (1 MJ of biodiesel, the functional unit considered in the sustainability evaluation) and the GHG emissions directly depends on the energy consumption in each process step.

4. Conclusions

This study evaluates the sustainability of biodiesel production from microalga *Chlamydomonas* sp. grown in 20 % (v/v) of brewery's wastewater, blended with pentose sugars. It is concluded that in terms of sustainability the sugars addition has no significant advantages. In terms of the analytical grade sugars addition effect, xylose promoted the highest lipid productivity (what was due to an increase in the microalgae growth rate) in the shortest growth time, although in terms of sustainability, measured by the indicators LCEE, FER and GW, it is the second best after ribose. However this difference between both sugars is not significant.

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