Life Cycle Assessment of Microalgae Production in a Sanitary Effluent Medium Supplemented with Glycerol

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Abstract: Microalgae are considered renewable and sustainable raw materials. They can be cultivated in wastewater, enabling its treatment for disposal into water bodies due to the sequestration of residual nutrients such as nitrogen and phosphorus. The biomass obtained can be used to develop biofuels and biosupplies. Recognizing the production potential of *Chlorella* sp. in an alternative culture media, this work aimed to evaluate the major environmental impacts on biomass production cultivated in three scenarios with NPK solution, effluent, and effluent with glycerol supplementation. Life Cycle Analysis was performed using data from a 20 L production scale. The most impacted categories in the process were the production of carcinogenic and non-carcinogenic pollutants, inorganic respiratory pollutants, ecotoxicity, land acidification, land occupation, global warming, and non-renewable energy use. Given the best environmental results, cultivation in effluent with glycerol supplementation led to fewer environmental impacts on *Chlorella* sp. cultivation since it showed higher biomass yield than the other two scenarios.

1 INTRODUCTION

Microalgae have been widely studied as a great potential raw material for the production of biofuels and biosupplies (Cheng et al. 2019, Do et al. 2022), e.g., biodiesel, dietary supplements, nutraceuticals, cosmetics, animal feeds, and pharmaceuticals (Molazadeh et al. 2019, Fawcett et al. 2022).

Microalgae also can grow in wastewater and produce low-cost biomass while removing or consuming organic and inorganic nutrients from wastewater, making them a sustainable alternative (Singh et al. 2020). These organisms offer economic and environmental advantages, including photosynthetic efficiency, high growth rate, and CO_2 sequestration (Muhammad et al. 2021, Li et al. 2022).

In addition, wastewater from various sources such as agriculture, households, and industries can contain anthropogenic pollutants that microalgae can remediate, which otherwise would pose risks to human health and the environment (Mofijur et al. 2021, Ahmed et al. 2022).

In this sense, microalgae's benefits to the environment are unquestionable since they can bring benefits during growth and biomass use. On the other hand, biomass production involves inputs and outputs that potentially impact each life cycle, related to the need for energy, inputs, equipment sanitization, separation, and drying. Thus, considering the entire process, there is an impact on the life cycle of industrial production of microalgae, whether in a conventional environment or with effluents. Life Cycle Assessment (LCA) is a valuable tool to

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determine the environmental impacts associated with the production of microalgae, evaluating emissions, energy, and resource consumption (Sun et al. 2019, Wu et al. 2019), highlighting critical points in the production process and comparing scenarios (Herrera et al. 2021).

In the context of the production of the microalgae *Chlorella* sp. in an effluent medium with and without glycerol (organic carbon source) supplementation, the effluent treatment was investigated, showing the benefit for the water treatment by this biological method and the importance of this biomass as a source of lipid (Amaral et al. 2022). This research seeks to recognize, from the experimental research already conducted, the potential environmental impacts of the production process in comparison with the use of NPK solution, an agricultural input widely used for microalgae biomass production.

2 SCOPE AND DELIMITATION OF THE SYSTEM

This LCA study aimed to quantify the potential environmental impacts of microalgae cultivation in sanitary effluent with and without glycerol supplementation, promoting the bioremediation of the effluent and enhancing the production of microalgal biomass compared to the production in NPK-rich medium. Thus, three scenarios were compared in this LCA research for *Chlorella* sp. production:

- Scenario 1: Microalgae cultivation in 3 g L^{-1} NPK solution according to Pacheco et al. (2019);
- Scenario 2: Microalgae cultivation in sewage effluent;
- Scenario 3: Microalgae cultivation in sanitary effluent with glycerol supplementation;

In all three scenarios, the steps were the same, using the same equipment for cultivation, harvesting, and drying. What differs one from the other is the culture medium and biomass yield for the same production duration.

The LCA of microalgal biomass production grown in sanitary effluent with and without glycerol supplementation was performed at bench-photobioreactor experiments (Figure 1) with sanitary effluent from the Wastewater Treatment Plant (WWTP/UNISC) obtained after anaerobic digestion. The NPK (Yara, 12:11:18) used was previously dissolved in water and filtered.

Figure 1. Photobioreactor used in microalgae cultivation.

When the microalgae reached a cell density of 4.75 x 10^5 cel mL^{-1}, they were cultivated in a 20 L photobioreactor with constant aeration and lighting. The experiment was terminated on day 10 (240 h). After cultivation, each sample was centrifuged individually in a benchtop centrifuge for 15 min at 2500 rpm, followed by drying for 24 h at 50 °C. The input and output data from each step, energy consumption, yield, inputs, and waste, were used in SimaPro version 8.5 software to conduct the Life Cycle Impacts Analysis (LCIA). The equipment used is shown in Table 1, and the process inputs and outputs are shown in Table 2.

The potential environmental impacts arising from the LCA for producing 1 kg of biomass considering cultivation, separation, and drying were evaluated in all three scenarios. All inputs and outputs were found in the Ecoinvent 3.6 database.

Table 1: Equipment used in the cultivation of microalgae in tubular photobioreactors.

Steps	Equipment	Specifications
Cultivation	Lamps	LED T8 9W 100-240V, ~ 50/60HZ
	Pumps	Aeration pump Vigo Ar/ 60Plus. AC 220V – 60Hz, 2.5 W, 90 L h^{-1},120 mbar.
Separation	Centrifuge	Centrifuge Sigma/ 6-16KS, Ano 2015, ~V/Hz 220-2 40/60, 2300 W.
Drying	Greenhouse	Greenhouse Tecnal/ TE-394, +7°C-70°C, 1.1 kW.

Table 2: Inputs and outputs of the three scenarios evaluated.

Item	Scenarios NPK	Wastewater	Wastewater + glycerol
Input			
N fertilizer (kg)	1.62	-	-
P fertilizer (kg)	1.48	-	-
K fertilizer (kg)	2.43	-	-
Wastewater (L)	20	20	20
Glycerol (g)	-	-	12.5
Water (L)	20	20	20
Clean Product (g)	2	2	2
Output			
Treated Effluent (L)	20	20	20

Comments: total values are shown in the table

3 LIFE CYCLE IMPACT ANALYSIS

In the study of environmental impacts in the three scenarios evaluated to produce microalgal biomass, it was observed that of the 15 categories presented by the Impact 2002+ method, the categories that are most significant in the process are related to the production of carcinogenic and non-carcinogenic pollutants, inorganic respiratory pollutants, ecotoxicity, and terrestrial acidification, land occupation, global warming, and non-renewable energy use. The impacts are associated with necessary inputs from the technosphere (water, detergent, glycerol, and NPK) and energy needs.

In the cultivation stage, there is a great influence of the use of electricity to operate the pumps to transfer CO_2 to the medium and to produce light energy, essential for photosynthesis and the conversion of inorganic carbon into biomass. In separating biomass by centrifugation and in oven drying, there was a greater contribution of impacts associated with electricity use.

By adding glycerol, the effect of electricity consumption was minimized due to the increased production of microalgal biomass, enabling production in a mixotrophic metabolic mode. Producing more biomass at the same energy consumption is a premise for achieving a lower environmental impact. Using inputs more efficiently leads to a higher biomass yield can reduce production's environmental impacts. Residual inputs can determine better yield without adding more economic and environmental impacts (Kabir et al. 2022).

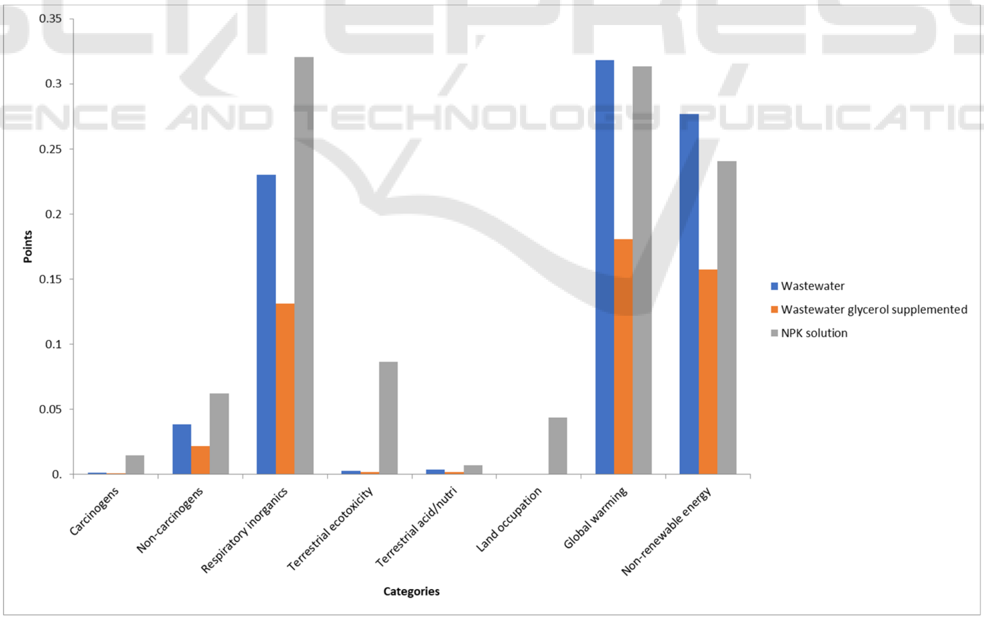

Figure 2. Impact categories obtained in the biomass production with different mediums (wastewater, wastewater supplemented with glycerol, and NPK solution) using Simapro 8.5 with Impact 2002+ method and Ecoinvent database.

It was observed that the glycerol supplementation scenario generated the least environmental impacts (Figure 2), even though purified glycerol was used as an input, which added environmental impacts to the system but led to a greater gain in biomass. Residual glycerol, obtained after methanol recovery, should have its pH analyzed and adjusted and, therefore, may be responsible for an even better environmental performance of microalgal biomass production. Crude glycerol is already recognized as a suitable carbon source in mixotrophic microalgae production (Xu et al. 2019, Gougoulias et al. 2022). In previous research conducted by this group, it was observed that adding glycerol may be a factor in improving the C/N ratio and using microalgae to reduce residual nitrogen after conventional urban wastewater treatment (not published). Figure 3 shows the main normalized environmental damages associated with microalgal biomass production from the scenarios under study.

The use of NPK in microalgae production adds the most impact in all categories, being the greatest for human health. Li et al. (2022), when studying the life cycle of microalgae production in wastewater using the ReCiPe method, which presents the impacts on mid and endpoints, also observed the benefits of using wastewater in microalgae production, showing a reduction in long-term impacts to human health and the ecosystem. They also noted the energy input requirement and no nutrient recovery effect as factors responsible for lower environmental performance.

In the uncertainty analysis for the three scenarios concerning the main impact categories, the data in Table 3 were obtained. The greatest uncertainties in the impact categories in both methods are related to the scenario in which microalgae was cultivated in a medium with NPK.

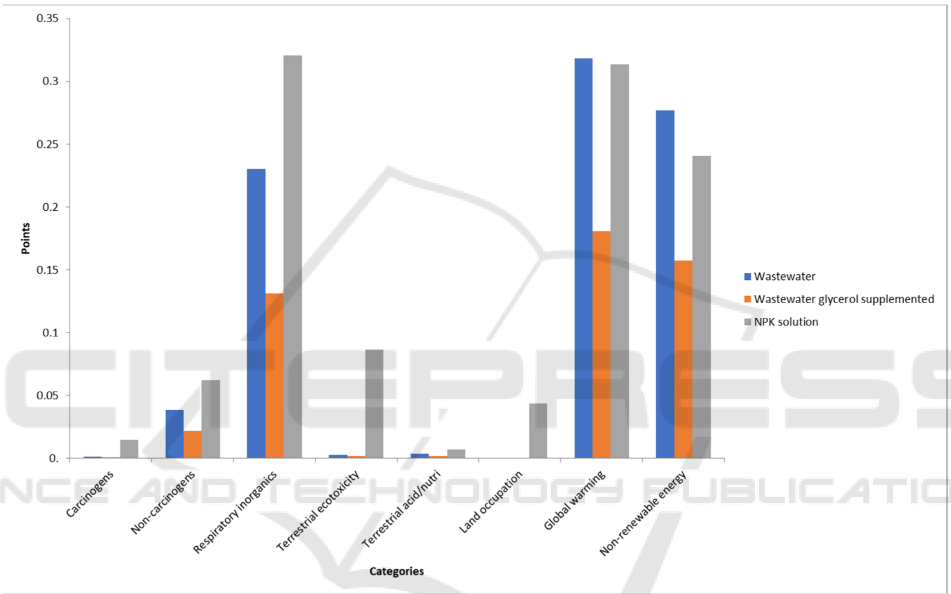

Figure 3. Environmental damage categories obtained in the biomass production with different mediums (wastewater, wastewater supplemented with glycerol, and NPK solution) using Simapro 8.5 with Impact 2002+ method and Ecoinvent database.

The uncertainties of these data for the main categories of impacts analyzed correspond to the division of the standard deviation by the mean in each category. The largest uncertainties in the impact categories in both methods are in the categories of land cover in the wastewater cultivation scenario and carcinogenic and non-carcinogenic pollutants for cultivation in NPK solution; however, all uncertainties were considered low since they are less than 0.3 (Pearson and Casarim 2018).

4 FUTURE PERSPECTIVES

For the production of *Chlorella* sp., besides the benefits associated with the fact that microalgae are photosynthesizers, responsible for carbon capture from the atmosphere, and are suitable for capturing nutrients from the water, it is observed by the Life Cycle Inventory (LCI) that they can also be promising as a cleaner production process if we use effluent for cultivation. Supplementing glycerol, especially crude glycerol, also adds a better environmental performance associated with a higher biomass yield.

To improve the process, it is still possible to reduce impacts associated with biomass drying by using a solar dryer with heated air through the biomass, as presented by Silva et al. (2021) for *Spirulina platensis*, which reached 11% of biomass moisture in less than 3.5 h.

Notably, the reduction of the impacts of the process by cultivating in an effluent medium with residual glycerol can lead to the loss of some properties and the risk of contamination, which reduces the possibilities of biomass use. This process can be environmentally cleaner, with several benefits. However, the use of the biomass obtained should be considered. Nevertheless, the biomass can be used as biofertilizer (Vishwakarma et al. 2022), and biofuels (de Souza Celente et al. 2019, de Souza et al. 2021) are more likely products to be developed with the biomass.

Another aspect of microalgae production's environmental viability is the cultivation location (de Souza et al. 2022). In this LCA, the need for effluent and glycerol transportation was not considered, and if there is transportation, the impacts increase due to fuel consumption. For many microalgae, the use of effluent in cultivation can be promising; however, if there are large distances between the generation of effluent and the production of microalgae, the alternative is no longer viable.

Table 3: Uncertainty analysis by Monte Carlo simulation (1000 interactions and 95% confidence) of the impact results of the categories highlighted in the LCIA with the Impact 2002+ method.

Categories	Unit	NPK		Wastewater		Wastewater + glycerol	
		Average	CV	Average	CV	Average	CV
Carcinogens	kg C_2H_3Cl eq	3.73E+01	0.183	3.70E+00	0.004	2.14	7.15E-03
Global warming	kg CO_2 eq	3.11E+03	0.036	3.15E+03	0.000	1791.28	1.67E-04
Land occupation	m^2org.arable	5.48E+02	0.033	5.22E-02	0.132	5.04	9.15E-02
Non-carcinogens	kg C_2H_3Cl eq	1.57E+02	0.120	9.72E+01	0.000	55.35	8.40E-04
Non-renewable energy	MJ primary	3.65E+04	0.036	4.21E+04	0.000	23906.24	1.46E-04
Respiratory inorganics	kg $PM_{2.5}$ eq	3.25E+00	0.047	2.33E+00	0.000	1.33	2.75E-04
Terrestrial acid/nutri	kg SO_2 eq	9.33E+01	0.042	4.80E+01	0.000	27.35	3.81E-04
Terrestrial ecotoxicity	kg TEG soil	1.50E+05	0.046	4.86E+03	0.002	3132.06	2.21E-02

5 CONCLUSIONS

The environmental performance of microalgal biomass production was obtained by LCA comparing three cultivation scenarios (NPK solution, effluent, and effluent with glycerol supplementation). When comparing the scenarios, it was observed that using sanitary effluent with glycerol supplementation led to less environmental impacts in the cultivation process of *Chlorella* sp. Thus, glycerol can reduce microalgal biomass production impacts when supplemented with effluent. However, this will depend on the biomass's applicability, production logistics, and transport impacts.

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