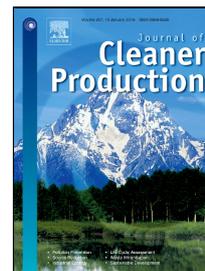


# Accepted Manuscript

Soil applications of microalgae for the recovery of nitrogen: a life-cycle approach

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PII: S0959-6526(18)33501-7  
DOI: 10.1016/j.jclepro.2018.11.097  
Reference: JCLP 14856  
To appear in: *Journal of Cleaner Production*  
Received Date: 12 April 2018  
Accepted Date: 09 November 2018

Please cite this article as: Mauro Henrique Batalha de Souza, Maria Lúcia Calijuri, Paula Peixoto Assemany, Jackeline de Siqueira Castro, Anna Carolina Martins de Oliveira, Soil applications of microalgae for the recovery of nitrogen: a life-cycle approach, *Journal of Cleaner Production* (2018), doi: 10.1016/j.jclepro.2018.11.097

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1 **Soil applications of microalgae for the recovery of nitrogen: a life-cycle**  
2 **approach**

3

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27 **Abstract**

28 The application of algal biomass in the soil represents an alternative of efficient use of fertilizers.  
29 In the present study, the environmental impacts generated by the application of 1 kg of nitrogen  
30 from the algal biomass (biofertilizer) were analyzed through life cycle analysis. Nitrogen was  
31 recovered from a meat processing industry effluent in a high-rate algal pond. Impacts related to  
32 the entire biofertilizer chain were mainly impacting on climate changes (115 kgCO<sub>2</sub>eq). Other  
33 categories (particle formation, terrestrial acidification, freshwater eutrophication and freshwater  
34 ecotoxicity) were not very representative. Biomass cultivation was the most critical step regarding  
35 energy and time consumption. On the other hand, the use of effluent as the culture medium for  
36 microalgae growth reduced impact categories, such as freshwater eutrophication. Results showed  
37 that microalgae cultivation and harvesting steps need to be technologically developed, especially  
38 when compared to a conventional fertilizer already established in the market. In order to make  
39 microalgae biofertilizer environmental advantageous, alternatives should be beforehand: i) the  
40 use of photovoltaic energy instead of hydropower energy; ii) the use of a nitrogen richer effluent;  
41 iii) and the consideration of an environmental compensation for the treatment of effluent can be  
42 accounted for, disregarding the biomass production stage.

43 **Keywords:** Nutrient recovery, biofertilizer, high rate algal ponds, life cycle analysis, algal  
44 biomass.

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## 56 1. INTRODUCTION

57 Nitrogen (N) is a resource obtained predominantly by the Haber-Bosch process.  
58 Approximately 80–90% of N synthesized by this process is used to produce fertilizers to grow  
59 more than half of the food consumed worldwide, making the process larger than the sum of all  
60 natural land processes combined (Galloway and Cowling, 2002, Galloway et al., 2008). The  
61 remaining N is used in the chemical industry for the production of nylon, plastics, explosives, and  
62 animal supplements (Galloway and Cowling, 2002; Galloway et al., 2008). The magnitude of  
63 human influence on N fluxes has substantially altered the natural N cycle, which has serious  
64 consequences on water resources (e. g. eutrophication), the lithosphere (saturation of N in the soil  
65 and impacts on biodiversity), the atmosphere (greenhouse gases - GHGs), acid rain, atmospheric  
66 pollution), and human systems (resource and economic restrictions). Although N is a renewable  
67 resource, the Haber-Bosch process is extremely energy demanding, accounting for approximately  
68 1% of the world's total energy consumption (Matassa et al., 2015).

69 With regard to environmental aspects, the use of any type (chemical or biological) of N  
70 fertilizers increases N content in the soil and favors the emission of nitrous oxide (N<sub>2</sub>O), an  
71 important GHG that affects global warming. In light of this, the Intergovernmental Panel on  
72 Climate Change has predicted that food production worldwide will suffer dramatic impacts in the  
73 coming decades due to the average increase in global temperatures (IPCC, 2007). Agriculture and  
74 livestock contribute to climate change as much as they suffer its consequences. Emissions of  
75 methane (CH<sub>4</sub>), carbon dioxide (CO<sub>2</sub>), carbon monoxide (CO), N<sub>2</sub>O, and nitrogen oxides (NO<sub>x</sub>),  
76 which are potential gases for global warming (GWP), have been generated by different  
77 agricultural practices and livestock management methods (Lima, 2002). Therefore, better soil  
78 management practices and the look for alternatives for the recycle of N from ecosystems, or at  
79 least to reduce the demand at the source, could help to minimize undesirable effects.

80 Among several sources of this nutrient, we highlight the effluents. Many industrial  
81 practices result in high nutrient levels and the nutrients may accumulate in ecosystems as a  
82 consequence of lack of treatment or inadequate treatment of these effluents. An alternative to the  
83 recycle, avoiding new exploitation, of these nutrients is the cultivation of microalgae associated  
84 with the treatment of wastewater. Although, it would not prevent N accumulation in ecosystems,  
85 it could allow a better management of the nutrient cycle.

86 Microalgae have evolved to assimilate nitrogen and phosphorus mainly in environments  
87 where nutrients are scarce, thus achieving high maximum specific absorption rates (Lehman and  
88 Scavia, 1982) and making these microorganisms extremely effective for use in biochemical  
89 processes of nutrient recovery. Specifically, microalgae are normally used for effluent polishing,  
90 i.e. for phosphorus, ammonia, and/or nitrate removals (Silva-Benavides and Torzillo, 2012;  
91 Shoener et al., 2014; Uggetti et al., 2014; Whitton et al., 2016). Microalgae also produce biomass

92 that can be used for fertilizers and bioenergy feedstock, providing additional revenue and  
93 improving the financial viability of the recovery method (Pittman et al., 2011). Castro et al. (2017)  
94 performed an experiment using microalgal biofilm as a nitrogen source for the cultivation of  
95 *Pennisetum glaucum*. Authors concluded that the establishment of a microalgal biofilm in the soil  
96 favored lower nitrogen loss through ammonia volatilization and increased organic matter content  
97 and cation exchange capacity in the soil. Additionally, the shoot dry matter mass production and  
98 N content assimilated by the plant were the same as those with urea fertilizer. Results were  
99 promising, indicating the potential of the microalgae biomass produced in effluents in the  
100 recovery of nutrients as a biofertilizer. However, as a new technology, information about its  
101 environmental and economical sustainability is still required.

102 An important tool to evaluate and account for the impact of microalgae production and  
103 valorization is the life-cycle assessment (LCA). According to ISO 14040 (2006), LCA is a  
104 methodology used to evaluate the environmental aspects associated to a product, being carried  
105 out through the collection and quantification of energy and materials needed for production,  
106 inputs of the system and waste and emissions released to the environment. It also supports decision-  
107 makers in industry, governmental or non-governmental organizations, allowing them to select  
108 indicators relevant to environmental performance, as well as marketing, giving the product eco-  
109 labeling or environmental statement. The results are dependent on the system boundary  
110 definitions, the database used for the processes, the efficiency of the processes and the functional  
111 units (Hill et al., 2006).

112 A problem with these types of studies is the lack of microalgae-producing facilities that  
113 operate under actual conditions and so, in many cases, extrapolation is required to estimate the  
114 performance of facilities based on real models. Consequently, a wide range of processes for  
115 microalgae production (cultivation systems, cultivated species, processes for harvesting, value  
116 products), results in a large variability of technical data. In addition, the methodological  
117 perspectives, which address the implementation stages of the LCA, such as functional units and  
118 system boundaries, are also widely variable (Collet et al., 2015). Different methodological choices  
119 cause discrepancies in current research results even when the technical data are similar  
120 (Benemann et al., 2012).

121 Therefore, the objective of this study was to evaluate and account for the impacts of the  
122 production, harvesting and application steps of microalgae biomass used as N fertilizer for the  
123 cultivation of millet (*Pennisetum glaucum*) and to compare the results to the impacts of using a  
124 traditional mineral fertilizer (urea). Experimental data obtained during the study of Castro et al.  
125 (2017) were thoroughly measured in order to supply the SIMAPRO® software and then, get real  
126 information about the sustainability in using microalgae biofilm as a biofertilizer. The LCA with  
127 primary and real experimentation data is the novelty of the work.

128

129 **2. MATERIALS AND METHODS**130 *2.1. Experimental configuration and primary data obtention*

131 A high rate algal pond (HRAP) (area = 3.3 m<sup>2</sup> and volume = 1 m<sup>3</sup>) operated in batch mode  
 132 (14 days of operation) was used to produced algal biomass. The effluent used as microalgae  
 133 culture medium (Table 1) was a primary pre-treated effluent collected after a flotation unit from  
 134 the wastewater treatment plant of a meat processing industry located in the city of Viçosa, Brazil.

135 Table 1. Wastewater characterization.

Variable	Values
pH	5.7
Volatile suspended solids (mg.L <sup>-1</sup> )	571.8
Total Kjeldahl nitrogen (mg.L <sup>-1</sup> )	68.4
Total phosphorus (mg.L <sup>-1</sup> )	7.4
Total chemical oxygen demand (mg.L <sup>-1</sup> )	1918.7

136 The HRAP had a two blades paddlewheels powered by 1HP electric motor responsible  
 137 for the operation of 12 ponds. Therefore, only one-twelfth of all spent energy was computed. A  
 138 CO<sub>2</sub> injection system was also used in order to control the pH of the pond, maintaining it between  
 139 7 and 8, thus providing greater productivity and N recovery. The addition of CO<sub>2</sub> was carried out  
 140 in the daytime using a gas cylinder containing 99% CO<sub>2</sub>. A 20W aquarium pump was used to  
 141 recirculate the effluent in the carbonation column. The average photosynthetically active radiation  
 142 (PAR) at 12 pm during the cultivation period was 1,445.4 ± 548 (μmol/m<sup>2</sup>.s).

143 After the production, the biomass was harvested using sodium hydroxide (NaOH) at 50%  
 144 m/v, promoting a pH increasing until 12. Paddlewheels were moved simultaneously for a short  
 145 period (approximately two hours), generating a hydraulic gradient favorable for coagulation and  
 146 promoting the sedimentation of the biomass, which was collected after resting the HRAP for 24  
 147 hours.

148 Two experimental plots of 4 m<sup>2</sup> were established and contained different treatments with  
 149 different N sources for the millet (*Pennisetum glaucum*) crop. The concentrated biomass was  
 150 applied manually in each plot in the soil. The treatments consisted of (i) 120 kg ha<sup>-1</sup> of N supplied  
 151 by algal biomass and (ii) 120 kg ha<sup>-1</sup> of N supplied by conventional urea. The experiment was  
 152 conducted over a period of 60 days in the winter. During the experiment, the closed chamber  
 153 method was used to measure the emissions of CH<sub>4</sub>, CO<sub>2</sub>, and N<sub>2</sub>O in each plot. Moreover,  
 154 volatilization of ammonia was determined according to Araújo (2009).

155 More details about the experimental methodology of the biomass production and  
 156 application in the soil can be found in Castro et al. (2017). As this paper does not aim to evaluate

157 microalgae effect in soil and plant, methodological and results aspects were limited to LCA  
 158 content. Table 2 presents a summary of the primary data used as entrance data in the LCA.

159 In each operation, 0.85 g/L of total kjeldahl nitrogen and 55 L of harvested biomass were  
 160 obtained. Therefore, 22.99 operations were needed in order to get 1 kg of nitrogen (used as the  
 161 system output unit for LCA). Each operation lasted 14 days (average time to get a decrease in the  
 162 algal growth, which was determined by daily monitoring of chlorophyll-*a*).

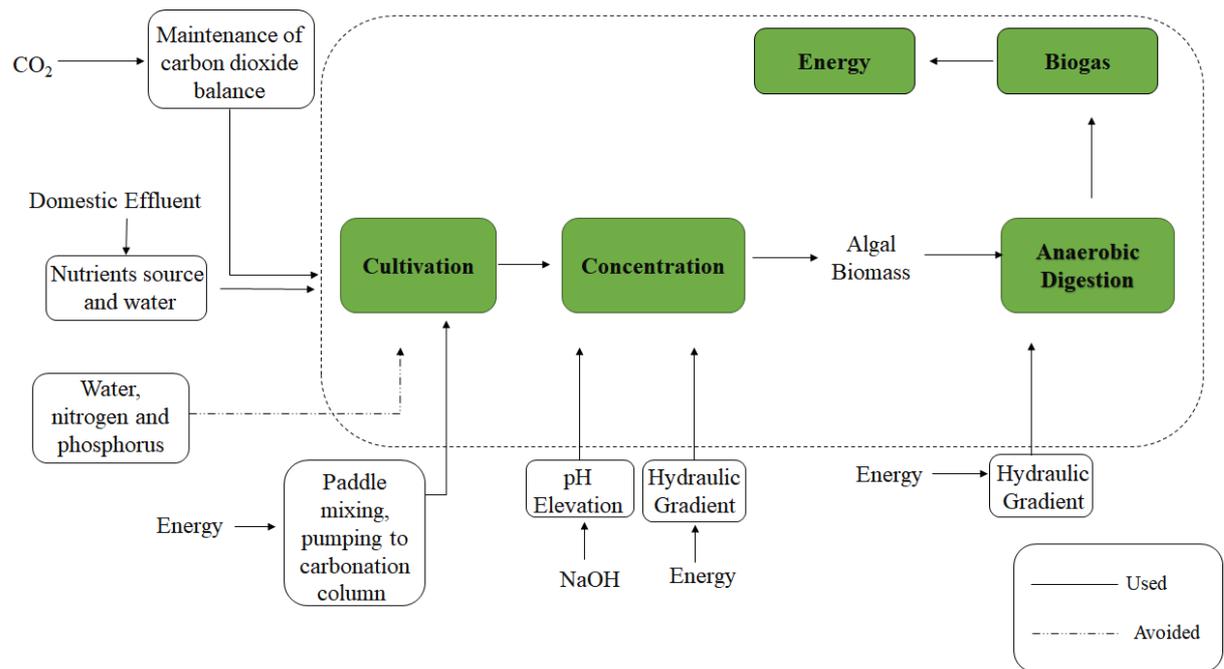
163 Table 2. Primary data used as entrance in the LCA.

	<b>Amount per operation</b>	<b>Time of operation</b>	<b>Power (kW)</b>	<b>Details</b>
<b>CO<sub>2</sub></b>	0.27 kg	20 min (4 times during 5 min each)	-	CO <sub>2</sub> Flux = 7 L/min 99% purity Density = 1.98 kg/m <sup>3</sup>
<b>NaOH</b>	1.515 kg	-	-	50% m/v Density = 1.515 kg/m <sup>3</sup>
<b>Paddlewheels motor</b>	-	24 h during 14 days	0.0613	-
<b>Carbonation column pump</b>	-	10 h during 14 days	0.02	-
<b>Pump for biomass concentration</b>	-	13 h	0.02	-

164 *2.2. LCA using SimaPro 8*

165 This study evaluated the energy consumption and impacts of the cultivation,  
 166 concentration, and application of the biomass in the soil. Figure 1 shows the boundaries of the  
 167 system used in the LCA.

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170 Figure 1. LCA system boundaries.

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172 SimaPro 8.1 software was used in order to quantify the impacts of the three analyzed  
 173 stages (biomass cultivation, harvesting, and soil application). Because the design of projects of  
 174 this size has a useful life of approximately twenty-five years, the construction phase was not taken  
 175 into account. A life-cycle impact assessment (LCIA) was conducted using the ReCiPe midpoint  
 176 methodology, which is focused on environmental issues, making it, therefore, the most qualified  
 177 method for this study (PRÉ, 2013). The midpoint method was used to prioritize short or medium  
 178 term impacts on a constant basis. The method has 18 impact categories, although some of them,  
 179 such as marine ecotoxicity and ionizing radiation, were not considered since they are not recurrent  
 180 in environmental impact studies related to effluent treatment and energy recovery.

181 The categories used in this study were climate change, terrestrial acidification, freshwater  
 182 eutrophication, human toxicity, terrestrial ecotoxicity, and particle formation. They were chosen  
 183 because they suffer direct impacts during the evaluated stages.

184 During the production stage, the inputs were the values of electric energy (kWh) used for  
 185 the paddlewheels rotation and the effluent recirculation pump into the carbonation column to add  
 186 the CO<sub>2</sub> into the HRAP. We assumed that water and nutrients were not inputs due to the use of  
 187 the effluent. In the biomass harvesting stage, the inputs were the volume of NaOH used as a  
 188 coagulant and the electric energy spent for maintaining the hydraulic gradient in the pond by  
 189 moving the paddlewheels for approximately two hours. It is important to note that there were two  
 190 distinct scenarios for the electric energy. The first was the use of hydroelectric energy and the  
 191 second was the use of photovoltaic solar energy. Finally, in the soil application stage, the inputs

192 were the measured volumes of GHGs. Table 3 presents a summary of the life-cycle inventories  
 193 (LCI) used and their units.

194 Table 3. LCI Inventory.

Stage			Unit	Description
<b>Biomass Production</b>	Input	Industrial Wastewater	m <sup>3</sup>	Provide nutrients and water for algal growth
		Electric Energy	kWh	Effluent recirculation through the carbonation column and paddlewheels operation
		CO <sub>2</sub>	kg	pH adjustment and CO <sub>2</sub> supply for biomass
	Avoided Products	Water	m <sup>3</sup>	Products that are no longer used once the cultivation has taken place in effluent
		Nitrogen Phosphorus	kg	
<b>Biomass concentration</b>	Input	Coagulant (NaOH)	kg	pH increasing
		Electric Energy	kWh	Promote hydraulic gradiente
<b>Soil Application</b>	Input	-		
	Output	CO <sub>2</sub>	kg	Gases emitted during the cultivation of millet
		CH <sub>4</sub>	kg	
		NO <sub>x</sub>	kg	
		NH <sub>3</sub>	kg	

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196 The inputs for the conventional fertilizer (urea) consisted of the production costs of the  
 197 fertilizer, which is available in the SimaPRO Software and is based on the Ecoinvent database  
 198 and the maritime and terrestrial transportation costs. Maritime transport is required because the  
 199 fertilizer originates in Russia and is imported to Brazil. Land transport was taken into  
 200 consideration because the ports where the fertilizers arrive are relatively far from the application  
 201 sites. In the soil application stage, the GHG volumes were measured in the same manner as the  
 202 GHG volumes from biofertilizer. One kg of N applied to the soil was used as the system output  
 203 unit.

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### 205 2.3. Possible scenarios for impact reduction

206 In order to minimize impacts, scenarios with different conditions to those applied in the  
 207 study were proposed: i) photovoltaic energy as an alternative source, instead of using hydropower  
 208 energy; ii) using other N-richer effluent; iii) environmental compensation for the treatment of  
 209 effluents, disregarding the biomass production stage.

210 Although considered by many as a renewable source of energy, hydroelectric power  
211 plants have a significant amount of impacts. Therefore, a scenario was proposed where all the  
212 energy used in the production and harvesting stages of the microalgae biomass (power supply for  
213 paddlewheels, effluent recirculation into the carbonation column and mixing after the use of the  
214 coagulant) was from a photovoltaic source. For this scenario, all components for the installation  
215 of a 3kWp photovoltaic plant, energy use for the mounting, transport of materials and persons to  
216 the construction place were considered, including the disposal of components after end of life.

217 In the scenario of using other N-richer effluent for culture medium, a double of initial N  
218 concentration in the effluent was considered and operation time was kept the same, 14 days.

219 Lastley, in recent years, the pressure from environmental organizations and  
220 environmental legislation has increased and has especially targeted different industries to ensure  
221 the treatment of effluents. For this scenario, all the impacts generated for the production stage  
222 were disregarded, considering that they come from a mandatory stage in any industry - the effluent  
223 treatment process. Therefore, the recovery of the nutrients would add value for the treatment,  
224 increasing environmental and economic benefits for the industry.

225

## 226 **RESULTS AND DISCUSSION**

### 227 *3.1. Life-cycle inventory*

228 Tables 4 and 5 show the LCI for the biofertilizer and uera, respectively. Biofertilizer  
229 results were based on experiments and, therefore, represent primary data, not literature-based  
230 data. Studies carried out in this manner are very important due to a shortage of data in these types  
231 of systems, especially in Brazil.

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245 Table 4. Biofertilizer life-cycle inventory.

Stage			Unity	Value
<b>Biomass Production</b>	Input	Industrial Wastewater	m <sup>3</sup>	22.99
		Electric Energy	kWh	531.36
		CO <sub>2</sub>	Kg	6.28
	Avoided Products	Water	m <sup>3</sup>	22.99
		Nitrogen	Kg	1.07
		Phosphorus	Kg	0.019
<b>Biomass harvesting</b>	Input	Coagulant (NaOH)	Kg	34.83
		Electric Energy	kWh	6.12
<b>Soil Application</b>	Input	-		
	Output	CO <sub>2</sub>	Kg	6.25 x10 <sup>-3</sup>
		CH <sub>4</sub>	Kg	-1.22x10 <sup>-6</sup>
		NO <sub>x</sub>	Kg	4.34 x10 <sup>-6</sup>
		NH <sub>3</sub>	Kg	4.63 x10 <sup>-5</sup>

246 Table 5. Urea life-cycle inventory.

Stage			Unity	Value
<b>Fertilizer Production</b>	Input	Ureia (46%)	Kg	2.17
<b>Transport</b>	Input	Maritime	Tkm*	20
		Terrestrial	Tkm*	0.8
<b>Soil Application</b>	Input	-		
	Output	CO <sub>2</sub>	kg	4.90 x10 <sup>-4</sup>
		CH <sub>4</sub>	kg	-2.72 x10 <sup>-6</sup>
		NO <sub>x</sub>	kg	0.11 x10 <sup>-6</sup>
		NH <sub>3</sub>	kg	1.89 x10 <sup>-4</sup>

247 \*Tonne-Kilometre: represents the transport of one tonne of products by a given transport over  
 248 one kilometer

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250 From Tables 4 and 5 it was possible to observe that values of electric energy, coagulant  
 251 and CO<sub>2</sub> were very high for producing 1 kg of N from the microalgae biomass. The energy use  
 252 can be attributed to the long operating time (14 days) of the HRAP. A slow algae growth was a  
 253 consequence of the effluent characteristics, mainly the high organic load (Table 1). On the other  
 254 hand, the use of a secondary treated effluent instead of a primary treated would result in lower or  
 255 no N recovery.

256 Urea consists of 46% N (Table 5) and it requires just over two kg of urea to obtain 1 kg  
257 of N. In addition, the N from Russia has to travel 20,000 km by ship to reach Brazil and another  
258 800 km by truck to the site application. Although the land transport mileage is high, Brazil is a  
259 very large country; therefore, such values are acceptable for transporting goods between ports and  
260 the main agricultural regions.

261 The gas emission values were negative for methane due to its absorption/oxidation at the  
262 source. This phenomenon occurs due to several factors such as soil type,  $\text{NH}_4^+$  concentration in  
263 the soil, soil moisture and temperature. Therefore, the inflow found in the field analyzes by Castro  
264 et al. (2017) was used as a compensation for GHG emissions. The high values of  $\text{CO}_2$  emitted by  
265 the biofilm when applied to the soil were due to the development of microorganisms other than  
266 microalgae in the soil, such as bacteria and fungi. Zhang and Wang (2005) and Zhang et al. (2007)  
267 stated that  $\text{CO}_2$  can be generated by the microbial decomposition of soil organic matter or  
268 respiration of plant roots and by microorganisms present in the soil. Similar results were observed  
269 for the  $\text{N}_2\text{O}$  emissions. Montes et al. (2014) reported that an important difference between mineral  
270 fertilizers and wastes, is that wastes contain organic carbon, which, depending on soil conditions,  
271 can affect  $\text{N}_2\text{O}$ . Marks et al. (2017) also founded that the microalgal suspension stimulated soil  
272  $\text{CO}_2$  production.

273 The lower volatilization of ammonia for the biofilm application may be related to the type  
274 of N that was applied to the soil; due to the slower degradation of the organic N, a slower nutrient  
275 release and fewer nutrient losses occurred.

### 276 3.2. Impact evaluations

#### 277 3.2.1. Impacts of the fertilizers

278 A comparison of the impacts of the system phases indicated that the biomass cultivation  
279 phase had the greatest impact in the climate change category of the ReCiPe method (Table 6).  
280 Perez-Lopez et al. (2014a; b) found that the major contributors to the impacts on microalgae  
281 production were electricity and the use of fertilizers. Despite the substitution of fertilizers by an  
282 effluent grown biomass in the present study, the consumption of electricity was much higher in  
283 comparison to other studies (Stephenson et al., 2010; Jorquera et al., 2010; Razon and Tan, 2011).  
284 The high energy consumption was probably due to the 22.99 operations of 14 days each that were  
285 needed to produce 1 kg of nitrogen. Moreover, the 1 cv motor used to run the paddlewheels was  
286 probably super estimated.

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290 Table 6. Impacts generated in the different phases of the process.

	<b>Impact Category</b>	<b>Climate Change</b>	<b>Particulate Matter Formation</b>	<b>Terrestrial Acidification</b>	<b>Freshwater Eutrophication</b>	<b>Freshwater Ecotoxicity</b>
	<b>Unity</b>	kgCO <sub>2</sub> eq	kgPM10 eq	kgSO <sub>2</sub> eq	kgP ep	kg1,4-DB eq
<b>Biofertilizer</b>	<b>Production</b>	107	0.156	0.297	0.0208	0.0338
	<b>Concentration</b>	17.22	0.0364	0.119	0.000366	0.01314
	<b>Soil Application</b>	-3.91x10 <sup>-6</sup>	0.0148	0.113	X	X
	<b>Total</b>	115.11	0.18	0.43	0.022	0.09
<b>Urea</b>	<b>Production</b>	7.38	0.02	0.06	1.01x10 <sup>-3</sup>	0.09
	<b>Land Transport</b>	0.27	4.40x10 <sup>-4</sup>	0.00	8.68x10 <sup>-7</sup>	3.03x10 <sup>-5</sup>
	<b>Marine Transport</b>	0.46	2.83x10 <sup>-3</sup>	0.01	4.86x10 <sup>-5</sup>	1.66x10 <sup>-3</sup>
	<b>Soil Application</b>	-6.07x10 <sup>-5</sup>	0.08	0.46	X	X
	<b>Total</b>	8.11	0.10	0.53	1.06x10 <sup>-3</sup>	0.09

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In addition to the high electricity consumption, the low total biomass production obtained in this study (570 mg/L) should be highlighted. The low productivity, together with the high hydraulic retention time of the HRAP (14 days) caused an increase in the inputs of the production stage. It is believed that the low growth of microalgae may be linked to the development of other microorganisms such as bacteria, fungi and protozoa. The use of the effluent resulted in competition, which hindered the growth of the microalgae.

The harvesting stage also had a large impact due to the use of NaOH as a coagulant. The coagulant NaOH was chosen because traditional coagulants such as ferric chloride and aluminum hydroxide could result in the bioaccumulation of metals in the millet. However, the quantity of the NaOH that was required to harvest the biomass was considerably larger than the amount for a traditional coagulant.

The stage of soil application had the lowest impact and zero impact occurred in half of the categories. In the climate change category, there was a negative impact, indicating that the soil application of microalgae is advantageous with regard to the effect on GHGs. According to the IPCC, methane is 25 times more polluting than CO<sub>2</sub>, which is mainly due to the absorption/oxidation of the methane in the soil.

Table 5 also shows the impacts related to the use of 2.17 kg of urea. This value was used since N represents 46% of the total mass of urea. In most cases, the production stage had a higher impact for urea due to high-energy system inputs (Júnior, 2011).

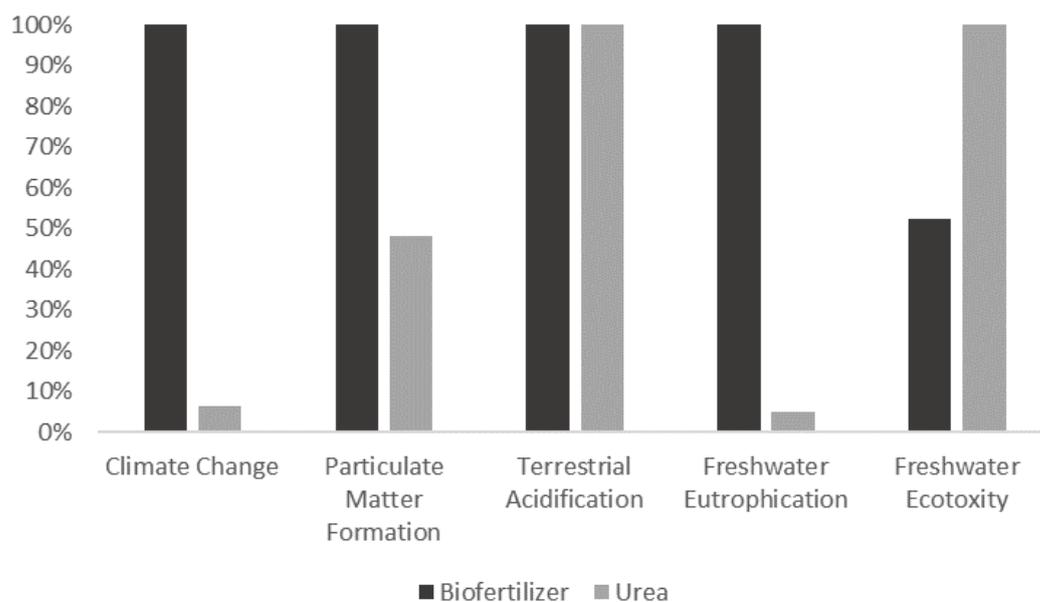
311 The transportation stages had little impact because the unit used in the software was tkm  
 312 (ton-kilometer). Since only 1 kg of N was used in this study, this value is insignificant, even with  
 313 high mileage. The values for the generation of gases were also low, similarly to the biofertilizer.

314 Arashiro et al. (2018) when comparing HRAP system for wastewater treatment where  
 315 microalgal biomass was reused for nutrients recovery through biofertilizer production reported  
 316 0.2 kg CO<sub>2</sub>eq, 0.015 kg SO<sub>2</sub>eq, 0.0025 kgPM10eq and 0.001 kPeq for the categories Climate  
 317 Change, Terrestrial Acidification, Freshwater Eutrophication and Particulate Matter Formation,  
 318 respectively. When comparing those impacts with a conventional technology for wastewater  
 319 treatment, authors highlighted higher impacts in Terrestrial Acidification, Particulate Matter  
 320 Formation, Human Toxicity and Terrestrial Ecotoxicity. NH<sub>3</sub> emissions to air derived from NH<sub>4</sub><sup>+</sup>  
 321 volatilization in HRAPs and heavy metals content in the biofertilizer were the main causes.

### 322 3.2.2. Comparison between biofertilizer and conventional fertilizer impacts

323 Figure 2 shows a comparison of the impacts (relative percentage) of using biofertilizer  
 324 and urea. Results showed that the conventional fertilizer had much less impact than the algae  
 325 biofertilizer except for terrestrial acidification and freshwater ecotoxicity. A comparison between  
 326 the subcategories showed that the gases generated in the soil application stage were higher for the  
 327 microalgae biofilm as mentioned earlier. In addition, the production stage was negatively  
 328 highlighted, increasing the differences between the types of fertilizers mainly in the categories  
 329 climate change and freshwater eutrophication.

330



331

332 Figure 2. Percentage comparison of impacts between urea and algal biofertilizer.

333 The results for the types of fertilizers were similar only in the terrestrial acidification  
 334 category, which was due to the high values of volatilized ammonia in the soil for the urea.  
 335 According to Goedkoop et al. (2009), ammonia has a high impact in this category.

336 In the context of using effluent as a fertilizer, Corbala-Robles et al. (2018) through a LCA  
 337 using the Recipe Midpoint methodology compared the application of pig manure effluent in the  
 338 soil with and without a previous treatment. The authors concluded that the untreated effluent had  
 339 the best environmental performance when compared to the treated effluent in 13 of 18 tested  
 340 categories. However, authors highlighted that the comparison between the treatment scenario and  
 341 the no-treatment scenario should be done with careful, as impacts at midpoint could be combined  
 342 into one final score using normalisation and weighing factors depending on the questions raised.

### 343 3.3. Potential ways of reducing impacts

#### 344 3.3.1. Source of energy

345 The results and the reductions/increases in comparison with hydroelectric power plants  
 346 source are shown in Table 7.

347 Table 7. LCI using photovoltaic energy.

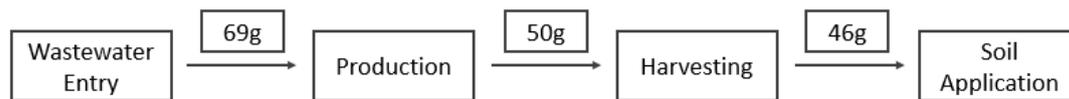
<b>Impact Category</b>	<b>Unity</b>	<b>Total</b>	<b>Reduction (%)</b>
Climate Change	kgCO <sub>2</sub> eq	31	73.07
Particulate Matter Formation	kgPM10 eq	0.080	56.27
Terrestrial Acidification	kgSO <sub>2</sub> eq	0.301	30.47
Freshwater Eutrophication	kgP ep	0.0272	-21.92
Freshwater Ecotoxicity	kg1,4-DB eq	4.99	-4930.24

348 Table 7 shows that the use of photovoltaic energy generates a large reduction in the impact  
 349 in most categories, mainly in the climate change category, where the largest impacts occurred.

350 In the categories eutrophication of water bodies and ecotoxicity of water bodies, the change  
 351 in the energy source resulted in a negative impact mainly due to the presence of several  
 352 carcinogenic heavy metals in the photovoltaic panels. Carcinogenicity is one of the main  
 353 parameters used in the calculation of the impacts and, therefore, has considerable weight  
 354 (Goedkoop et al., 2009).

#### 355 3.3.2. Effluent choice

356 The N balance based on the Kjeldahl method is shown in Figure 3. It is known that there  
 357 are losses in all stages of the process but they were minimized in this study to obtain an efficiency  
 358 greater than 66% in the recovery of all N introduced into the system. An eventual automation of  
 359 some of these processes may result in even smaller losses in future studies, thus increasing the  
 360 efficiency of the N recovery.



361

362 Figure 3. Nitrogen mass balance

363 The losses in the production stage occurred mainly because the pH control was performed  
 364 manually, which caused some failures. The loss related to the harvesting step was less than 5%  
 365 and was associated with biomass lost in the clarified effluent.

366 It is important to note that, even if there were no losses in the system, the mass of N  
 367 introduced into the system was small and several operations were required to obtain 1 kg of N.  
 368 This result indicates that the meat processing industry effluent may not be the most suitable type  
 369 for nutrient recovery by biomass soil application as a biofertilizer considering the entire process  
 370 of batch production in the HRAPs. We suggested that a continuous operation should be  
 371 performed, resulting in low energy input and increasing yields over time, moreover a nitrogen-  
 372 richer effluent should be tested for biomass cultivation. Table 8 presents impacts simulation if a  
 373 nitrogen-richer effluent was used.

374

375 Table 8. LCI using a nitrogen-richer effluent.

	<b>Climate Change</b>	<b>Particulate Matter Formation</b>	<b>Terrestrial Acidification</b>	<b>Freshwater Eutrophication</b>	<b>Freshwater Ecotoxicity</b>
<b>Unity</b>	kgCO <sub>2</sub> eq	kgPM10 eq	kgSO <sub>2</sub> eq	kgP ep	kg1,4-DB eq
<b>Production</b>	46.59	0.070	0.121	0.010	0.014
<b>Reduction (%)</b>	56	55	59	54	57
<b>Concentration</b>	8.61	0.0182	0.0595	0.000183	0.00657
<b>Reduction (%)</b>	50	50	50	50	50

376

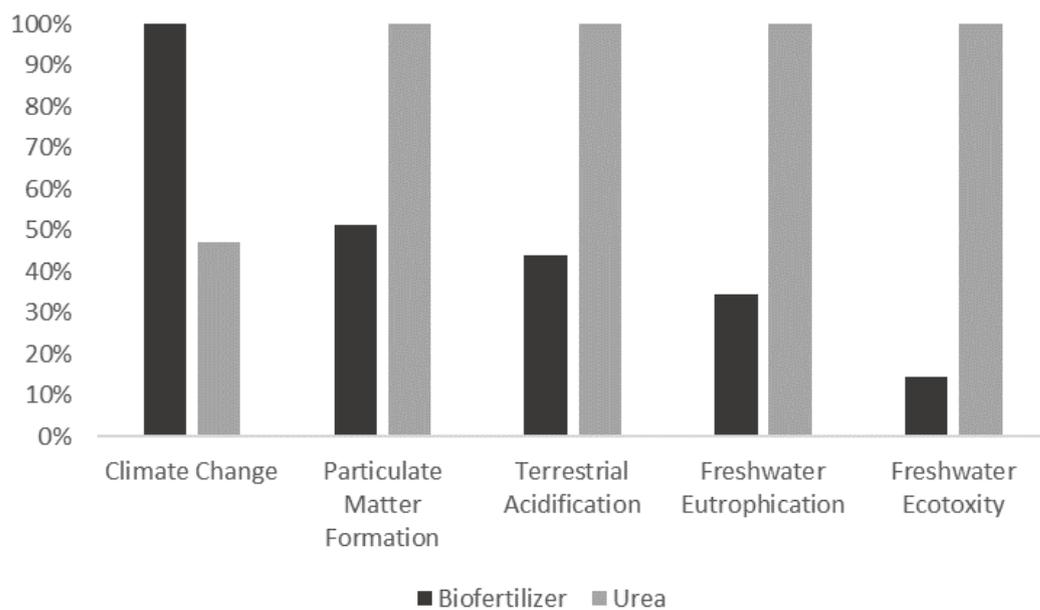
377 Simulating twice the concentration of nitrogen in the effluent, the number of operations  
 378 was halved. The avoided products – the avoided nutrient discharge in the water courses –  
 379 remained the same, however, was obtained more than 50% of impact reduction in all the  
 380 categories when considering the biomass production phase. Results indicated that the net gain  
 381 was positive, probably due to energy lower requirement with lower operation period. For the  
 382 biomass concentration phase 50% less impact was observed in all categories.

383 Prior to choosing the route of recovery/valorization of the biomass (biofuels,  
 384 biofertilizers, among others), the effluent must be evaluated with regard to the best use of the  
 385 biomass considering its characteristics. In this study, effluents with higher concentrations of N  
 386 would be more suitable. However, it is important to note that a pre-treatment should be considered

387 depending on the characteristics of the effluent and special attention should be given to the  
388 ammonia toxicity to microalgae growth.

### 389 3.3.3. Environmental compensation

390 Figure 4 shows the results for the case of not accounting for the production stage. In this  
391 scenario, the biofertilizer was preferred over urea in terms of environmental impacts because only  
392 an insignificant amount of electric energy was needed to harvest the biomass. The climate change  
393 was the only impact category that the biofertilizer had a higher impact, compared with the urea.



394  
395 Figure 4. Percentage comparison between fertilizers excluding the biomass production stage.

### 396 3.4. Potential limitations of the study

397 This study compared the impacts of using a biofertilizer derived from microalgae and a  
398 conventional fertilizer (urea). Although we considered impacts inherent to fertilizers, it should be  
399 emphasized that urea is a fertilizer that is established in the market while the biofertilizer  
400 represents a relatively new technology. The scale in production step and gaps in the hole process,  
401 such as the algal biomass harvesting, resulted in greater impacts for the biofertilizer. It is  
402 important to note that many studies have shown that different types of fertilizers (urea, ammonium  
403 nitrate, ammonium sulfate, triple superphosphate, etc.) have different efficiencies and are  
404 recommended accordingly in specific situations (Júnior, 2011). For the biofertilizer used in this  
405 study, the variability is related to the composition of the biomass, which is influenced by the type  
406 of effluent, the timing in production, and other factors. Addressing these challenges will require  
407 further study.

408 In LCA studies, some impacts should be considered to act locally (eg, terrestrial  
409 acidification) and others globally (eg, climate change). This will affect the choice between the  
410 proposed scenarios. However, the objective of comparing scenarios is to provide a solid scientific  
411 basis for the environmental consequences of choosing either option. LCA is not sufficiently  
412 developed to present a clear conclusion of these choices (Schaubroeck et al., 2015). Schaubroeck  
413 and Rugani (2017) suggested that additional research is needed to improve LCA studies and also  
414 to integrate the social and economic impacts of the proposed studies.

415 However, even if all the previous considerations were applied, at an environmental point  
416 of view, some impacts such as the climate change, have no well defined frontiers. Therefore, the  
417 discussion of local charges is made difficult.

418 Recently, an application of LCA tool in regions or territories was introduced (Loiseau et  
419 al., 2014) and may be of interest. While in the traditional approach, impacts mainly linked to a  
420 pre-established functional unit are calculated, the territorial approach associates the study with  
421 the area. It delivers as a result beyond the environmental impacts, functions of land use value,  
422 such as meeting the needs of a population or wealth creation. Nevertheless, the new methodology  
423 makes some LCA steps more complex, especially choosing the boundaries of the system and  
424 obtaining data that reflect regional impacts.

425 The evaluation of the use of microalgae as biofertilizers presents a focus on the  
426 environmental sustainability. However, other aspects can be accounted for (eg. socioeconomic),  
427 and different methods of calculating the impacts can be used, such as the Recipe Midpoint used  
428 in the present study. As for socioeconomic impacts, for example, it can be argued that, thanks to  
429 the treatment of effluents, there can be incentives and even increase the profitability of rural  
430 producers who use such practices.

#### 431 4. CONCLUSIONS

432 The main contribution of this study is to evaluate the potential of microalgae biofertilizer  
433 and to use LCA tool to identify the key issues, which can serve to guide the development of new  
434 technologies and to make them more competitive when compared to traditional fertilizers.

435 An important result is that the more diffusion of new less environmentally impacting  
436 technologies than the use of environmental compensation can be considered to minimize the  
437 impact generated by the production of biofertilizers from algae, thus increasing their  
438 competitiveness with traditional fertilizers. The choice of the effluent based on the value of the  
439 biomass is another factor of great importance, especially considering that the biomass  
440 characteristics will vary depending on the environment in which it was cultivated. In addition, the  
441 type of harvesting system had a large impact and studies of continuous cultivation are required to  
442 determine approaches to minimize these impacts.

443 Further research is needed to optimize the algae production chain and to determine the  
444 possibility of obtaining higher value products. At the same time, cost analyses must be performed  
445 because aside from environmental impacts, economic factors must be taken into account to choose  
446 the best alternative.

## 447 5. ACKNOWLEDGEMENTS

448 The authors gratefully acknowledge the Brazilian National Council for Scientific and  
449 Technological Development, CNPq (grant number 404027/2013-0) and the Foundation for  
450 Research Support of the State of Minas Gerais, FAPEMIG (grant number APQ – 03160-13) for  
451 their financial support.

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## HIGHLIGHTS

- This study evaluated microalgae biofertilizer in a life cycle assessment
- Nitrogen was recovered from a meat processing industry effluent
- Biomass cultivation was the most critical step
- Impacts related to the biofertilizer chain were mainly impacting on climate changes
- Life cycle as a tool to guide the development of new and more competitive products