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LCA FOR AGRICULTURE PRACTICES AND BIOBASED INDUSTRIAL PRODUCTS

Life cycle assessment of bacterial cellulose production

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Abstract

Purpose Bacterial cellulose (BC), obtained by fermentation, is an innovative and promising material with a broad spectrum of potential applications. Despite the increasing efforts towards its industrialization, a deeper understanding of the environmental impact related to the BC production process is still required. This work aimed at quantifying the environmental, health, and resource depletion impacts related to a production of BC.

Methods An attributional life cycle assessment (LCA) was applied to a process design of production of BC, by static culture, following a cradle-to-gate approach. The LCA was modeled with GaBi Pro Software using the ReCiPe 2016 (H) methodology with environmental impact indicators at midpoint level. The functional unit was defined as 1 kg of BC (dry mass), in 138.8 kg of water.

Results From the total used resources (38.9 ton/kg of BC), water is the main one (36.1 ton/kg of BC), most of which (98%) is returned to fresh waters after treatment. The production of raw materials consumed 17.8 ton of water/kg of BC, 13.8 ton/kg of BC of which was for the production of carton packaging, culture medium raw materials, and sodium hydroxide (for the washing of BC). The remaining consumed water was mainly for the fermentation (3.9 ton/kg) and downstream process (7.7 ton/kg). From the identified potential environmental impacts, the production of raw materials had the highest impact, mainly on "Climate change", "Fossil depletion", "Human toxicity, non-cancer", and "Terrestrial toxicity". The sodium dihydrogen phosphate production, used in the culture medium, showed the highest environmental impacts in "Human toxicity, non-cancer" and "Terrestrial ecotoxicity", followed by corn syrup and carton production. The static culture fermentation and downstream process showed impact in "Climate change" and "Fossil depletion".

Conclusions Per se, the BC production process had a small contribution to the consumption of resources and environmental impact of the BC global life cycle.

Keywords Bacterial cellulose · LCA · ReCiPe 2016 · Climate change · Energy consumption · Water consumption

1 Introduction

Microcrystalline cellulose, micro/nanofibers, and nanocrystals from vegetable cellulose have many potential applications related to a wide range of industrial needs, while meeting the society demand for more environmentally-friendly materials. Worldwide, several manufacturing facilities are currently producing these celluloses in pre-commercial and commercial scale. Such companies include Stora Enso, Nippon Paper, American Process, Borregaard, and UPM Kymmene Corporation. Exilva, Borregaard's microfibrillated cellulose, is manufactured in the first commercial production facility in the world, with a capacity of 1000 ton/year (dry basis) (Hjørnevik 2018). However, the production of nanocelluloses involves high capital investment, the use of various chemicals, and mechanical methods with intensive

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use of energy (Esa et al. 2014; Soykeabkaew et al. 2017). An alternative source of cellulose is bacterial cellulose (BC), a nanofibrillar exopolysaccharide produced mainly by Gram-negative acetic acid bacteria, the *Komagataeibacter* genus being the most important due to the high cellulose yield obtained from a wide range of carbon/nitrogen sources (Lee et al. 2014). BC has several unique physicochemical and mechanical properties, like high purity, high crystallinity, high degree of polymerization (Ashori et al. 2012), ultrafine fibril network, high water holding and water absorbing abilities (Saibuatong and Phisalaphong 2010), high tensile strength in the wet state (Lejeune and Deprez 2010), and the possibility to be shaped into three-dimensional (3D) structures during synthesis. Due to its unique properties, this biopolymer has been studied in several applications, including biomedicine, textile, pulp and paper, (bio)composites, electronic paper displays, cosmetics, and in food applications (Klemm et al. 2011, 2001; Chawla et al. 2009; Müller et al. 2013; Nimeskern et al. 2013; Esa et al. 2014; Lee et al. 2014; Shi et al. 2014; Rajwade et al. 2015; Jozala et al. 2016).

Several fermentation technologies have been experimented using specific fermentation media, overproducing mutant strains and different bioreactors (Chawla et al. 2009; Pertile et al. 2010; Shah et al. 2013; Keshk 2014; Lee et al. 2014). However, the large-scale BC production remains a challenge due to the low productivity rates, ineffective fermentation systems, high capital investment, and high operating costs (Jozala et al. 2015, 2016; Campano et al. 2016). Consequently, the product BC has been mainly used in two applications: (i) in the food industry, usually employed as a food product (for example, “nata de coco”) mostly produced through traditional fermentation methods and consumed in Asian countries, and (ii) in high-value-added niche markets such as medical applications and for cosmetic industry (Dourado et al. 2016a).

With the increasing environmental awareness worldwide, companies are encouraged to design “greener” processes and products. The industrial biotechnology is especially emphasized on reductions of environmental impacts and risks, particularly in terms of climate change and fossil resource depletion, envisaging new economic viable and low impact bioproducts and bioprocesses (Fröhling and Hiete 2020; Mussatto et al. 2015). Although the current production worldwide is quite small as compared to the plant cellulose-based industries, assessing the environmental impacts of BC production may lead to better options in process design and optimization, considering the massive production for different market applications, while considering also the environmental sustainability (Sukara and Meliawati 2016; Ullah et al. 2016).

In previous work, the techno-economic feasibility of the BC production process (Dourado et al. 2016a), the market potential (Gama and Dourado 2018; Dourado et al. 2016b) and the key aspects in the regulatory framework (Dourado et al. 2016c) pertaining to the commercialization of BC have been addressed. The collected data was used in this work, for the development of an LCA study (Dourado et al. 2016a), allowing to quantify the environmental impacts through a cradle-to-gate analysis.

LCA has increasingly been used for the measurement of the environmental impacts related to the production of several bio-based products including plant cellulose, nanocellulose, and cellulose nanocrystals (Hervy et al. 2015; González et al. 2011; Li et al. 2013; Arvidsson et al. 2015; Gu et al. 2015; Shatkin and Kim 2015). Recently, Silva et al. (2020) performed a comparative LCA study on lab-scale BC fermentation using ILCD 2011 Midpoint V1.05 (Hauschild et al. 2011) and explored the impact of different culture media composition. Both production and LCA studies were carried over an industrial scale simulated process (Silva et al. 2020). A simple, lab-scale LCA of BC production comparing two different culture media was also done by Aragão et al. (2020). However, to the best of our knowledge, no LCA studies on the BC production using ReCiPe 2016 methodology have been published.

From the above, a thorough understanding of the environmental impact of the BC production process, covering the whole life cycle of BC, is of paramount importance to evaluate its environmental sustainability. The scope of this study is set for a cradle-to-gate analysis, and the system boundary covers from the raw material extraction to the production and transport of raw materials used in bacterial cellulose chain process, the production of BC, all the transportations and the wastewater treatment.

2 Methodology

2.1 Description of the BC production process

As depicted in Fig. 1, the simulated industrial BC production process published in (Dourado et al. 2016a) is divided into 4 stages (Fig. 1):

1. “Culture Medium Preparation”, which includes the culture medium preparation and pasteurization;
2. “Inoculum Propagation”, aiming to increase biomass, performed in two sequential batch fermenters (100 L and 1000 L);
3. “Static Culture Fermentation”, the main phase of the process, where the fermentation occurs under static culture conditions, in a clean room at 30 °C, for 7 days;

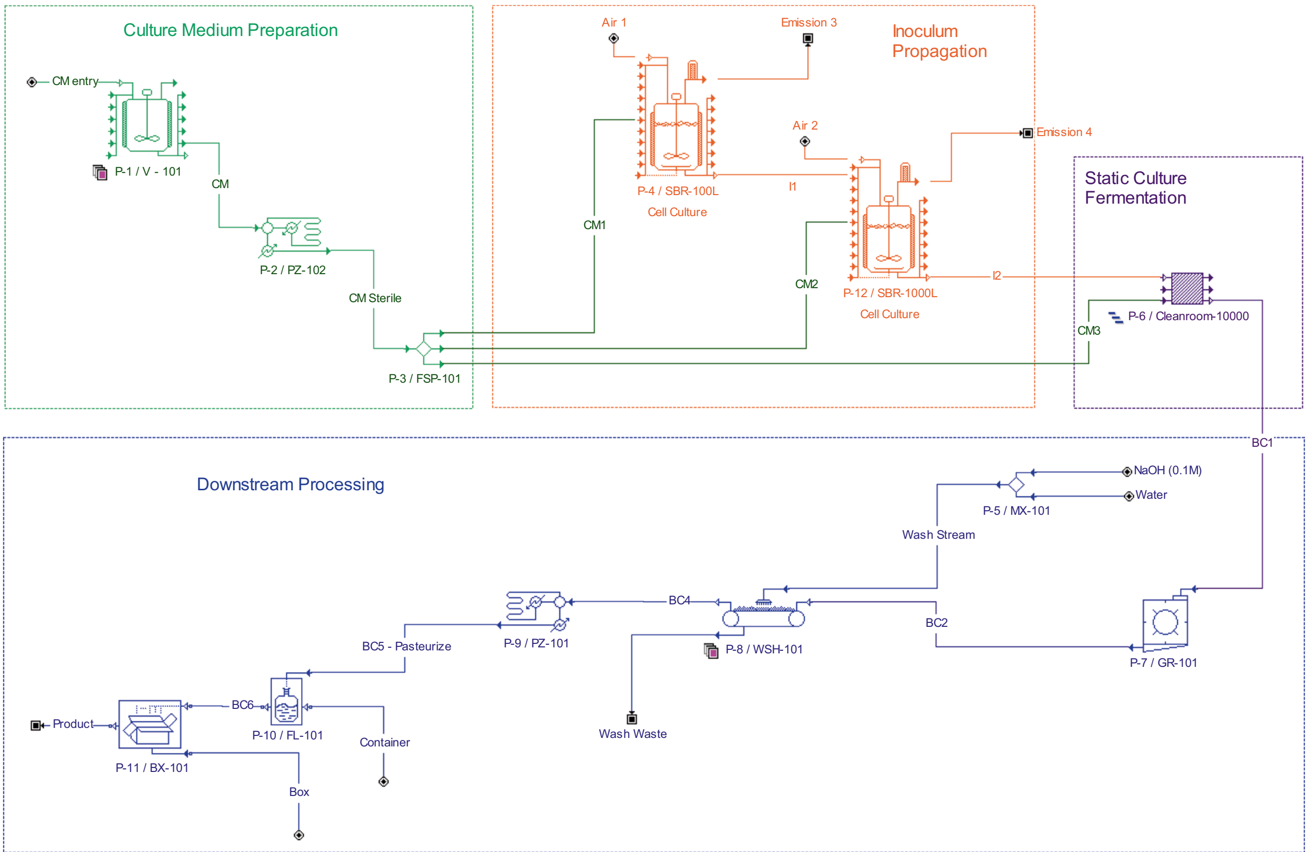

Fig. 1 Flowsheet (process design in SuperPro Designer) of the BC process chain dived into 4 stages: culture medium preparation (green), inoculum propagation (orange), static culture fermentation (purple), and downstream process (blue)

4. "Downstream Process", involves the purification of the BC into the final product (washed and ground cellulose, packed in plastic containers and carton boxes).

The plant was designed to process 60,000 L/month of culture media. With a BC production yield of 7 g/L (dry basis), this production volume yields 420 kg/month, i.e., 5 ton/year of dry product (Dourado et al. 2016a).

Inoculum propagation is usually achieved by successive propagation of biomass at a ratio of 1:10 (biomass/culture media). For the sake of simplicity, propagations bellow 100 L were omitted in the design, as, comparatively, these represent a very low volume. As such, two seed fermenters with 100 (SBR-100 L) and 1000 L (SBR-1000 L) capacities were considered for biomass growth ("Inoculum Propagation" stage). A single entry containing the mixture of the culture medium components was fed to a storage tank (V-101) before pasteurization (PZ-102). The pasteurized culture medium was then sequentially fed to each of the seed fermenters. Each seed fermenter operated for 3 days. The bacteria and additional pasteurized culture medium (up to a total volume of 10,000 L) were then combined and transported to a "cleanroom" for the fermentation under static conditions. This "generic unit" represents a controlled environment room with a minimum level of pollutants, operating at 30 ºC for 7 days, to simulate static culture conditions. The resulting BC sheets were collected, cut into cubes (GR-101), and washed with sodium hydroxide and water (WSH-101). The cubes were then pasteurized and packed (in plastic bags and cardboard boxes; FL-101 and BX-101, respectively) and stored ("Downstream Processing" stage).

2.2 Goal and scope definition and description of system boundaries

In western countries, bacterial cellulose is not yet produced at large scale. The goal of this study was to quantify the environmental impacts of the process, guiding the design of commercial scale for future BC production towards the minimization of environmental impacts. We aim at ascertaining whether BC may represent a more sustainable source of cellulose, e.g., as an alternative to cotton production that heavily relies on the use of pesticides and abundant use of water. This work aims at laying the foundation for such comparison studies.

The functional unit was defined as 1 kg of BC (bone-dry mass), in 138.8 kg water, with a consistency of 0.72%. This LCA studied the material and energy flows from the extraction of natural resources and their transformation to the production of BC, including the treatment and disposal of the produced waste from the BC process chain. A cradle-to-gate perspective is particularly relevant for materials that have many downstream applications, some of which have not yet been fully developed. Cradle-to-gate LCA results can then be used in subsequent cradle-to-grave LCA studies for the products in which the produced material, in this case BC, is one constituent (Arvidsson et al. 2015) of downstream applications. Figure 2 presents the system boundary and process stages for the cradle-to-gate LCA of BC.

For the life cycle impact assessment (LCIA) study, alongside with the four stages of the BC production process (Fig. 1), three additional stages were considered: the wastewater treatment for the liquid effluent produced, the production, and the transportation of raw materials used in BC production (Fig. 2).

For each stage, energy and mass balances were calculated using Gabi Pro software (version 9.2.1.68), considering all the flows from each process, and then categorized based on the type of used resource (energetic or material) for input flows, and the residues' disposal site in the terms of output flows. Unless otherwise stated, the raw materials, energy and water inputs and outputs are reported based on the production of 1 kg of BC (dry mass).

2.3 Inventory analysis

The data used to model the BC production process chain, i.e., the foreground system, was taken from (Dourado et al. 2016a), while for the background system data (regarding energy resources, extraction, transformation, and transporting materials), the Ecoinvent database from Gabi Pro software (previously ThinkStep, now Sphera) was used. The concept adopted in this work incorporates our experience in the fermentation and downstream processing of BC to a Technology Readiness Level of 4–5. Table 1 presents the data related to the electricity use, based on the data provided by (Dourado et al. 2016a) and other, retrieved from the Internet (Table S1).

Table 1 Equipment's electric power consumption during the whole process of BC production

Equipment	kWh
Mixer—culture medium preparation	3
Pasteurizer—1st pasteurization	15
Belt press filter—cellulose washing	90
Reactors—inoculum propagation (100 L)	75
Grinder—cellulose grinding	144
Pasteurizer—2nd pasteurization	30
Filler—filling plastic container machine	3
Packer—boxing	0.5

Data related with the raw materials (cells, culture medium reactants) for biomass growth (inoculum) and corresponding CO_2 emissions from aerobic fermentation were neglected due to their negligible contribution to the whole process. Data related to the equipment and raw materials used in foreground system was collected from (Dourado et al. 2016a), the literature, and from suppliers.

Regarding the fermentation method, static culture was selected. As for the culture medium, low-cost substrates were chosen for this study. The relevant inventory data (Table 2) was obtained from Keshk et al. (2006).

The estimated distances used to model the transport of raw materials were based in specific materials suppliers'

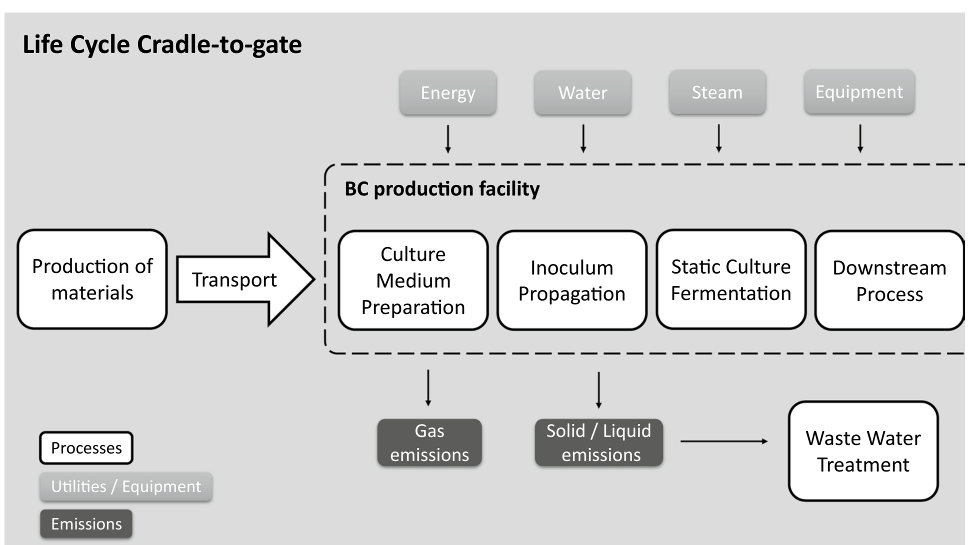

Fig. 2 Cradle-to-gate life cycle assessment system boundary

Table 2 Culture medium components

Culture medium component	Mass (kg/batch)	Mass kg/kg BC (dry mass)
Sugar beet molasses	221.03	3.03
Citric acid	11.05	0.15
Corn syrup	110.52	1.51
Sodium dihydrogen phosphate	33.15	0.45
Water	10,675.85	146.26

locations (Table 4—"Production and transport of raw materials" and Table S2), in relation to a hypothetical BC production facility located in Braga, Portugal. The distance was calculated using Google Maps. The raw materials transported to the factory include the culture medium components and sodium hydroxide solution used to wash BC and others (Table 4). Briefly, using the low-cost substrate (Table 2), BC was produced with a yield of 7 g/L (dry mass), following a 7-day static culture fermentation process. In this process, after washing, one BC fermentation batch produces a mixture of 10,131 kg of water with 0.72% BC. The final product is packed in a plastic (high-density polyethylene, HDPE) container and finally in a carton box. Data for equipment use, input and output flows, utilities, cooling water, and steam use in the BC production are available in the supplementary information (Tables S1, S2, S3, and S4).

The generated wastewater was processed in a treatment plant. Data for the wastewater is shown in Table 3. The effluent's organic load complies with the quality water standards from the municipal wastewater treatment company (AGERE—Empresa de Águas Efluentes e Resíduos de Braga, E.M., Portugal).

Using Gabi wastewater LCI datasets, a proxy for our wastewater processing unit was made assuming a standard European municipal wastewater treatment facility, where 50% of the sludge is processed by sludge incineration and 50% by an agricultural application (used as fertilizer).

The electricity input for the production of BC was assessed using the Portuguese average electricity grid mix (which includes coal, wind energy, natural gas, and hydroelectric power). This LCA considers only the essential equipment needed for each process, as shown in Table 4 and Table S1. The lifetime of these equipments was assumed to be of 10 years, equivalent to 2518 working days. Each polypropylene tray used in the static culture fermentation holds 2.5 L of culture medium. For each fermentation batch, 4000 trays are necessary.

Table 3 Characterization of the wastewater from the BC production (from da Silva et al. 2020)

Parameter	Value (g L^{-1})
Suspended solids	20.6
Volatile solids	13.5
Total nitrogen	0.90
Sulfates (SO_4^{2-})	1.83

2.4 Impact assessment

LCA was modeled using Gabi Pro software (version 9.2.1.68) and the impact assessment method ReCiPe 2016 (Huijbregts et al. 2017), which converts the emissions (gaseous and liquid) and the depletion of natural resources into 18 mid-point impact categories. For the simulation of the BC production process life cycle, using Gabi Pro software, the environmental impact was calculated for each life cycle stage of the BC production (Fig. 1). The details are provided in the supplementary information (Figs. S1, S2, S3, S4, S5 and S6).

For comparison of the data here obtained with the work of Silva et al. (2020), the environmental impacts were also estimated using ILCD 2011 Midpoint V1.06 (Hauschild et al. 2011). The results presented in the referred study were converted to kg (dry BC).

2.5 Sensitivity analysis

A sensitivity analysis was used to evaluate the impact of three parameters (inputs): transport distance (scenario 1), electricity consumption (scenario 2) and cooling water in the BC process chain (scenario 3), by increasing each of these parameters by 50%. The amount of water used for the culture medium and preparation of sodium hydroxide solution (to wash BC) were not considered, because these were already optimized; further changing the water content in the culture medium (while maintaining the composition of the culture medium) would impact on the absolute amount of produced BC, sizing of the equipment and BC facility and other variables. From this variation, the environmental impacts (output) were recalculated using the output of original scenario and output of the scenario considered:

$$Variation(\%) = \frac{Output_{original\ value} - Output_{scenario}}{Output_{scenario}} \times 100$$

3 Results and discussion

3.1 Mass balance

Table 5 summarizes the mass balance of input (resources) and output (deposited goods and emissions) flows of the BC life cycle, per kg of dry BC. The total used resources

Table 4 Input and outputs for the several processes and their auxiliary processes. Values are reported to the functional unit (1 kg BC dry basis)

Life cycle stage	Process	Input/output	Description	Quantity	Unit	Auxiliary process
Production and transport of raw materials (Fig. S2)	Transport of sodium dihydrogen phosphate	Input	RER: sodium phosphate, at plant	0.45[a]	kg	Sodium Dihydrogen Phosphate Production[g] (Database name: "RER: sodium phosphate production")
		Input	Transport	1068.78[b]	kg km	RER: Small lorry (7.5t) incl. fuel ELCD[h]
	Transport of molasses	Input	Sugar beet molasses	3.03[a]	kg	Market for molasses, from sugar beet[g]
		Input	Transport	169.53[b]	kg km	RER: Small lorry (7.5t) incl. fuel ELCD[h]
	Transport of citric acid	Input	Citric acid	0.15[a]	kg	GLO: Citric acid production[g]
		Input	Transport	409.71[b]	kg km	RER: Small lorry (7.5t) incl. fuel ELCD[h]
	Transport of corn syrup	Input	Corn Syrup	1.51[a]	kg	Corn Syrup Production[g]
		Input	Transport	531.44[b]	kg km	RER: Small lorry (7.5t) incl. fuel ELCD[h]
	Transport of sodium hydroxide	Input	Sodium hydroxide (100%)	1.11[a)]	kg	EU-28: Sodium hydroxide (caustic soda) mix (100%) ts[h]
		Input	Transport	110.59[b]	kg km	RER: Small lorry (7.5t) incl. fuel ELCD[h]
	Transport of plastic	Input	Polyethylene high density granulate (HDPE/PE-HD)	0.21[a]	kg	RER: Polyethylene high density granulate (PE-HD) ELCD/PlasticsEurope[h]
		Input	Transport	6.58[b]	kg km	RER: Small lorry (7.5t) incl. fuel ELCD[h]
	Transport of cardboard	Input	Cardboard (packaging)	1.59[a]	kg	Carton Production[h] (Database name: "RoW: carton board box production service, with offset printing")
		Input	Transport	28.62[b]	kg km	RER: Small lorry (7.5t) incl. fuel ELCD[h]
	EU-28: Tap water from groundwater	Input	Water (public tap water)	563.41[a, h]	kg	
Culture medium (CM) preparation (Fig. S3)	Mixer	Input	Citric acid	0.15[a]	kg	
		Input	Corn syrup	1.51[a]	kg	
		Input	Electricity	0.15[c]	MJ	PT: Electricity grid mix[h]
		Input	Sugar beet molasses	3.03[a]	kg	
		Input	Sodium Dihydrogen Phosphate	0.45[a]	kg	
		Input	Steel part	0.08[d]	kg	GLO: Manufacturing (stainless steel product)[h]; DE: Steel billet (100Cr6)[h]
		Input	Water (processed)	42.47[c]	kg	
		Input	Water (tap water)	146.26[e]	kg	

Table 4 (continued)

Life cycle stage	Process	Input/output	Description	Quantity	Unit	Auxiliary process
		Output	Culture Medium	151.41	kg	
		Output	Water (wastewater, untreated, CM Preparation)	42.47[f]	kg	
	Pasteurization 1	Input	Culture Medium	151.41	kg	
		Input	Electricity	1.0[c]	MJ	PT: Electricity grid mix[h]
		Input	Steam (vlp)	4.0[c]	kg	GLO: Steam conversion (vlp)[h]; PT: Process steam from natural gas 95%[h]
		Input	Steel part	0.01[d]	kg	GLO: Manufacturing (stainless steel product)[h]; DE: Steel billet (100Cr6)[h]
		Input	Water (tap water)	851.41[c]	kg	EU-28: Tap water from groundwater[h]
		Output	Culture Medium	151.41	kg	
		Output	Water (processed)	42.47	kg	
		Output	Water (tap water)	146.26[e]	kg	
		Output	Water (wastewater, untreated, CM Preparation)	662.69[f]	kg	
Inoculum propagation (Fig. S4)	Inoculum propagation	Input	Culture Medium	14.76	kg	
		Input	Electricity	7.0[c]	MJ	PT: Electricity grid mix[h]
		Input	Steel part	0.01[d]	kg	GLO: Manufacturing (stainless steel product)[h]; DE: Steel billet (100Cr6)[h]
		Input	Water (tap water)	96.28[c]	kg	EU-28: Tap water from groundwater[h]
		Input	Bacterium	0.01	kg	PT: Production of biomass[h]
		Output	Culture Medium Inoculum	14.77	kg	
		Output	Water (wastewater, untreated, Inoculum Propagation)	96.28[f]	kg	
Static culture fermentation (Fig. S5)	Static culture fermentation	Input	Culture Medium	136.64	kg	
		Input	Culture Medium Inoculum	14.77	kg	
		Input	Electricity	9.0[c]	MJ	PT: Electricity grid mix[h]
		Input	Polypropylene (PP) injection moulding	0.05[d]	kg	RER: PP injection molding part Plastics Europe[h]
		Input	Steam (vlp)	5.96[c]	kg	GLO: Steam conversion (vlp)[h]; PT: Process steam from natural gas 95%[h]
		Input	Water (processed)	7.0[c]	kg	
		Output	BC in water	137	kg	
		Output	Water (wastewater, untreated)	4.75	kg	

Table 4 (continued)

Life cycle stage	Process	Input/output	Description	Quantity	Unit	Auxiliary process
		Output	Water (wastewater, untreated, Fermentation)	6.60[f]	kg	
Downstream process (Fig. S6)	Cellulose grinding	Input	BC in water	137	kg	
		Input	Electricity	7.10[c]	MJ	PT: Electricity grid mix[h]
		Input	Steel part	0.01[d]	kg	GLO: Manufacturing (stainless steel product)[h] DE: Steel billet (100Cr6)[h]
		Input	Water (wastewater, untreated)	4.75	kg	
		Output	BC in water	137	kg	
		Output	Water (wastewater, untreated)	4.75	kg	
	Cellulose washing	Input	BC in water	137	kg	
		Input	Electricity	4.44[c]	MJ	PT: Electricity grid mix[h]
		Input	PT: Sodium Hydroxide (0.05 M)	564.51	kg	
		Input	Steel part	0.01[d]	kg	GLO: Manufacturing (stainless steel product)[h]; DE: Steel billet (100Cr6)[h]
		Input	Water (wastewater, untreated)	4.75	kg	
		Output	BC in water	137.0	kg	
		Output	Wastewater (bacterial cellulose washing)	569.26[f]	kg	
	Pasteurization 2	Input	BC in water	137.0	kg	
		Input	Electricity	1.48[c]	MJ	PT: Electricity grid mix[h]
		Input	Steam (vlp)	5.58[c]	kg	GLO: Steam conversion (vlp)[h]; PT: Process steam from natural gas 95%[h]
		Input	Steel part	0.005[d]	kg	GLO: Manufacturing (stainless steel product)[h]; DE: Steel billet (100Cr6)[h]
		Input	Water (tap water)	1136.27[c]	kg	EU-28: Tap water from groundwater[h]
		Output	BC in water	137.0	kg	
		Output	Water (processed)	6.60	kg	
		Output	Water (wastewater, untreated, Downstream Process)	1129.66[f]	kg	
	Filling plastic container	Input	BC in water	137.21	kg	
		Input	Electricity	1.18[c]	MJ	PT: Electricity grid mix[h]
		Input	Polyethylene high density part (HDPE/PE-HD)	0.206[a]	kg	

Table 4 (continued)

Life cycle stage	Process	Input/output	Description	Quantity	Unit	Auxiliary process
		Input	Steel part	0.01[d]	kg	GLO: Manufacturing (stainless steel product)[h]; DE: Steel billet (100Cr6)[h]
		Output	BC in water (in plastic bag)	137.21	kg	
	Boxing	Input	Aluminium part	0.002[d]	kg	PT: Electricity grid mix ts[h]; EU-28: Aluminum ingot mix[h]; DE: Thermal energy from natural gas[h]
		Input	BC in water (in plastic)	137.21	kg	
		Input	Cardboard (packaging)	1.59[a]	kg	
		Input	Electricity	0.20[c]	MJ	PT: Electricity grid mix[h]
		Output	Final Product Cellulose	138.80	kg	
Municipal wastewater treatment	EU-28: Municipal wastewater treatment (mix)	Input	Wastewater	2506.97[h]	kg	

[a] Raw materials used in the culture medium and for packaging, more details in Table S2

[b] Calculated based on the distance (km) between the products supplier and the city of Braga, Portugal (Table S2). Value estimated using "Google Maps" and by considering the weight of the material transported per functional unit produced. Table S2 in supplementary material details the values used in calculations

[c] Electricity, steam, and cooling water used by the process equipment based on SuperPro Designer simulation and Google search. Table S3 in supplementary material details the values used in calculations

[d] Essential equipment to the BC process chain, expressed in kg/working day/functional unit. Table S4 in supplementary material details the values used in calculations

[e] Part of the cooling water used in the Pasteurization 1 is reused as water in the culture medium, to save water

[f] Wastewater from different processes (in total 2506.97 kg/functional unit)

[g] Process data from Ecoinvent database (version 3.3)

[h] Process data from Gabi professional database (version 8.7)

Table 5 Mass balance of input (resources) and output (deposited goods and emissions) flows of the BC life cycle (1 kg BC, dry mass)

	Total (kg)	Production of raw materials (%)	Transport of raw materials (%)	Culture medium preparation (%)	Inoculum propagation (%)	Static culture fermentation (%)	Downstream process (%)	Wastewater treatment (%)
Energy resources	7.311	58.3	1.4	5.0	4.0	12.6	14.2	4.4
Material resources	38,856.509	45.9	0.0	3.8	8.5	9.9	19.9	12.0
Non-renewable elements	0.384	81.9	0.0	5.7	0.6	0.9	3.1	7.8
Non-renewable resources	20.554	31.8	0.1	8.1	5.6	11.0	19.4	24.1
Renewable resources	38,835.571	45.9	0.0	3.8	8.5	9.9	19.9	12.0
Water	36,129.812	49.3	0.0	4.0	9.1	10.7	21.3	5.7
Other renewable resources	2705.759	0.8	0.0	0.8	0.3	0.5	1.5	96.1
Resources (Total)	38,863.819	45.9	0.0	3.8	8.5	9.9	19.9	12.0
Deposited goods	13.473	25.3	0.1	7.7	6.8	7.6	19.2	33.4
Emissions to air	296.037	29.9	0.1	5.4	11.2	18.2	27.2	8.1
Emissions to fresh water	35,860.994	47.9	0.0	1.6	8.8	10.6	18.1	12.9
Analytical measures to fresh water	0.165	39.3	0.0	0.5	0.9	1.1	2.2	56.0
Ecoinvent long-term to fresh water	0.399	100.0	0.0	0.0	0.0	0.0	0.0	0.0
Inorganic emissions to fresh water	0.286	73.1	0.2	0.9	1.6	2.1	4.0	18.0
Other emissions to fresh water	35,554.288	47.9	0.0	1.6	8.9	10.7	18.2	12.8
Radioactive emissions to fresh water	305.776	50.3	0.3	2.1	4.2	5.0	10.2	28.0
Emissions to sea water	58.171	21.0	0.0	11.5	5.3	23.4	26.9	11.8
Emission (total)	72,089.683	47.8	0.0	1.7	8.8	10.7	18.2	12.9

Mainly water; related to the production of energy and transport and production of raw materials

were about 38.9 ton/kg of BC, including both energy and material ones, within which about 21 kg/kg BC were from non-renewable resources (such as chromium, tungsten, etc.), and 38.9 ton/kg BC from renewable resources, mainly water (36.1 ton/kg of BC). The production of raw materials consumed a total of 17.8 ton of water/kg of BC, of which 13.8 ton/kg of BC to produce carton used for packaging, culture medium raw materials, and sodium hydroxide (for the washing of BC). The remaining water was consumed in the fermentation (3.9 ton/kg of BC) and downstream process (7.7 ton/kg of BC). The overall results in Table 5 clearly highlight the large quantitative contribution of the activities related to the production of raw materials both in terms of consumption of resources and emissions (more than 50% of the global process).

Except for the wastewater process, production and transportation of raw materials, all other stages of the life cycle rely on one or several electricity generation processes (modeled based on Portuguese average electricity grid mix) to power the machinery, including hydroelectricity. Together, these processes consume a total 13.5 ton of water/kg to generate electricity, 13.4 ton/kg of which being emitted to fresh water.

The wastewater treatment consumed almost 4.7 ton/kg of renewable resources (water and air), 2.05 ton/kg of which corresponding to process water and emitted 4.6 ton/kg to fresh water. This difference can be explained because the purpose of this stage is to convert the BC process chain wastewater (calculated as 2.45 ton/kg) into "clean" water, whereby additional water was used for the treatment process. From these results, water amounts to 93% of the total resources used, being treated back to fresh water. Overall, only 0.46 tons/kg of water was consumed.

3.2 Energy consumption

Figure 3 displays the energy consumption (per functional unit) as obtained from data on Table 4. The figure shows that, in almost equal proportions, the highest amount of energy consumption out of a total value of 31.6 MJ/kg BC occurs during the static culture fermentation (27%), the inoculum propagation (21%), and the BC grinding process (22%), followed by the washing process (14%).

3.3 Environmental impact assessment

The environmental impacts from cradle-to-gate are presented in Table 6. They were calculated using the ReCiPe 2016 Midpoint (H) methodology. These results show that the life cycle stage of production of raw materials has the highest contribution in most of the mid-point categories of the environmental impact. Specifically, it impacts on "Climate change", "Fossil depletion", "Human toxicity, non-cancer", and "Terrestrial toxicity". These impacts were associated mostly to corn syrup production, followed by, in the respective order, disodium phosphate, sodium hydroxide, polyethylene, and carton production (Table 6) (Fig. S2). The sodium dihydrogen phosphate production represents the larger share of the environmental impacts measured in the impact categories "Human toxicity, non-cancer"

Fig. 3 Energy consumption for the whole life cycle of BC production. Values are based on the functional unit of 1 kg BC dry basis)

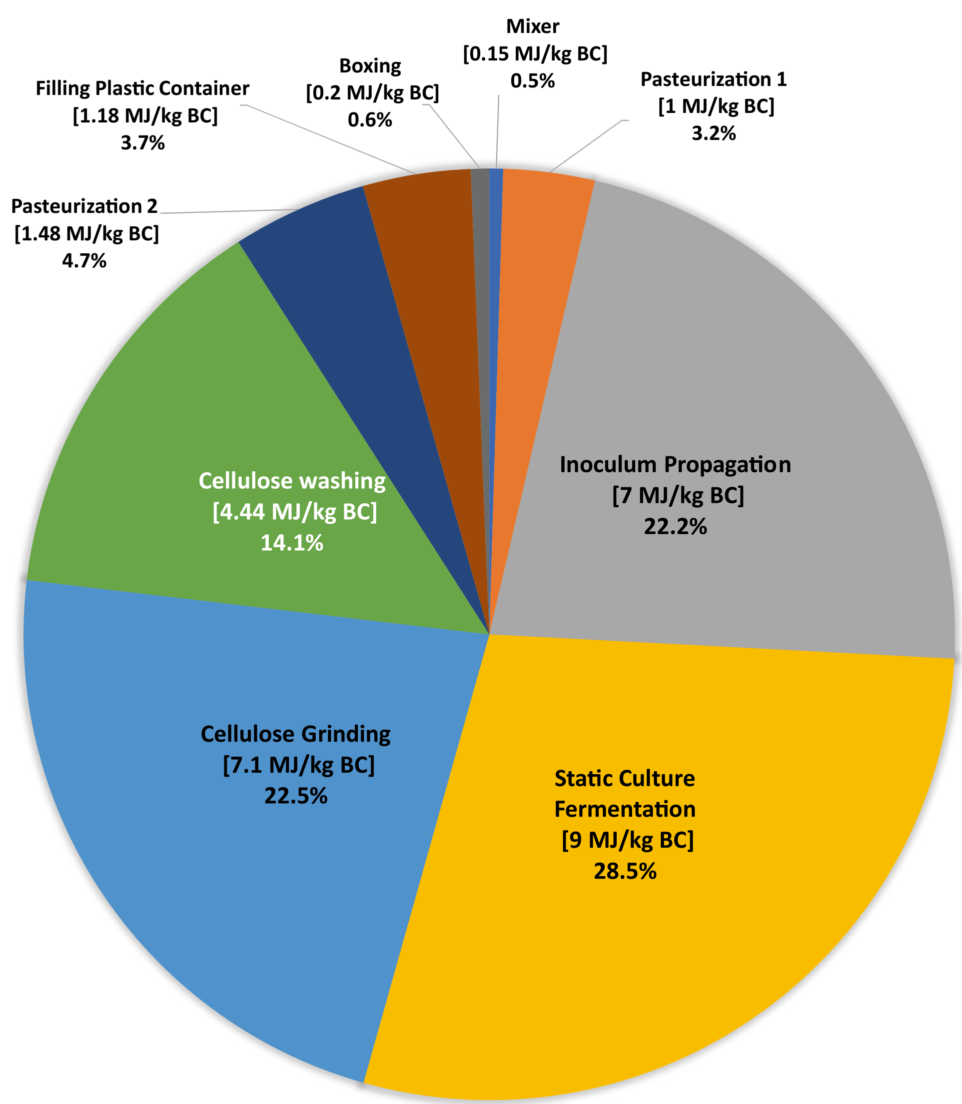

Table 6 Environmental impacts of life cycle of BC using ReCiPe 2016 Midpoint (H) for 1kg of dried BC, blue bars represent positive values while red bars represent negative values

Impact categories	Units	Total	Production of raw materials	Transport of raw materials	Culture Medium Preparation	Inoculum Propagation	Static Culture Fermentation	Downstream Process	Wastewater treatment
Climate change, default, excl. biogenic carbon.	[kg CO_2 eq.]	16.774	7.350E+00	3.200E-01	1.030E+00	8.330E-01	2.670E+00	2.940E+00	1.620E+00
Climate change, incl. biogenic carbon	[kg CO_2 eq.]	16.729	6.410E+00	3.200E-01	1.030E+00	8.310E-01	2.670E+00	2.940E+00	2.520E+00
Fine particulate matter formation	[kg PM2.5 eq.]	0.016	1.234E-02	3.580E-04	2.830E-04	3.250E-04	8.370E-04	9.130E-04	6.420E-04
Fossil depletion	[kg oil eq.]	6.565	3.785E+00	1.050E-01	3.610E-01	2.340E-01	8.880E-01	9.380E-01	2.560E-01
Freshwater consumption	[m^3]	0.470	7.740E-01	2.800E-05	8.590E-01	1.170E-01	3.290E-02	1.180E+00	-2.500E+00
Freshwater ecotoxicity	[kg 1,4-DB eq.]	0.086	5.999E-02	9.450E-06	1.820E-04	5.360E-05	5.020E-05	3.000E-04	2.580E-02
Freshwater eutrophication	[kg P eq.]	0.004	2.390E-03	5.290E-08	1.020E-05	2.870E-06	7.480E-06	1.670E-05	1.550E-03
Human toxicity, cancer	[kg 1,4-DB eq.]	0.826	6.930E-01	9.260E-06	1.250E-03	6.600E-04	1.250E-03	2.730E-03	1.280E-01
Human toxicity, non-cancer	[kg 1,4-DB eq.]	13.765	7.648E+00	2.090E-03	4.640E-02	1.730E-02	1.700E-02	8.080E-02	5.950E+00
Ionizing radiation	[Bq C-60 eq. to air]	0.342	3.009E-01	6.460E-05	1.550E-03	2.540E-03	2.940E-03	6.610E-03	2.680E-02
Land use	[Annual crop eq.·y]	0.967	6.260E-01	0.000E+00	1.370E-02	7.030E-02	8.420E-02	1.400E-01	3.320E-02
Marine ecotoxicity	[kg 1,4-DB eq.]	0.123	8.563E-02	2.680E-04	3.380E-04	1.670E-04	1.840E-04	6.290E-04	3.530E-02
Marine eutrophication	[kg N eq.]	0.004	1.020E-03	4.080E-07	2.230E-05	1.740E-05	1.920E-05	5.630E-05	2.950E-03
Metal depletion	[kg Cu eq.]	0.113	6.388E-02	1.540E-05	2.400E-02	3.160E-03	1.230E-03	2.760E-02	-7.000E-03
Photochemical ozone formation, Ecosystems	[kg NOx eq.]	0.030	1.746E-02	2.440E-03	9.900E-04	1.100E-03	2.840E-03	3.280E-03	2.070E-03
Photochemical ozone formation, Human Health	[kg NOx eq.]	0.029	1.628E-02	2.420E-03	9.700E-04	1.090E-03	2.800E-03	3.240E-03	2.050E-03
Stratospheric ozone depletion	[kg CFC-11 eq.]	0.000	5.375E-06	7.530E-08	4.700E-07	2.930E-07	9.450E-07	1.200E-06	6.990E-06
Terrestrial acidification	[kg SO_2 eq.]	0.043	3.229E-02	1.010E-03	8.150E-04	1.010E-03	2.650E-03	2.790E-03	2.000E-03
Terrestrial ecotoxicity	[kg 1,4-DB eq.]	15.625	1.427E+01	3.170E-02	1.800E-01	1.530E-01	1.870E-01	3.660E-01	3.950E-01

and "Terrestrial ecotoxicity", followed by corn syrup and carton production. These production processes generate pesticides, heavy metals, and other emissions to soil, water, and air and may cause damage to the ecosystem, and/or accumulate in the food chain, eventually affecting humans (Huijbregts et al. 2017).

The static culture fermentation and downstream process stages (Fig. S6) impacted most in both categories of "Climate change" and "Fossil depletion", mainly due to electricity consumption (50.7% of the total energy, Fig. 3). The equipment (Table 1) with the highest electric total power consumption are, in decreasing order, the grinder (GR-101), the washer (WSH-101), the pasteurizer (PZ-101), the filler (FL-101), and the packaging machine (BX-101).

The wastewater treatment process is responsible for the significant part of impacts in the categories "Climate change, including biogenic carbon" and "Human toxicity, non-cancer" (this is the risk increase of non-cancer disease incidence, through the accumulation of chemicals in the human food chain). A negative value was obtained for "Freshwater Consumption" category since the wastewater from the BC process chain is treated and discharged back to the water distribution network. As referred before, in this stage, the amount of treated water is higher than that of the consumed fresh water.

The present results were compared to the results obtained for the production of BC in the work by Silva et al. (2020), a cradle-to-gate life cycle assessment. The authors assessed the environmental impact of several culture media, both at laboratorial and industrial scale design, using the ILCD 2011 Midpoint V1.05 methodology (Hauschild et al. 2011). For comparison purposes, their results were converted to the same functional unit, 1 kg of BC (dry basis), as used in this work. In addition, this assessment was carried on by using the closest version available for the impact assessment methodology, i.e., ILCD 2011 Midpoint V1.06 (Hauschild et al. 2011). Table 7 compares the estimated impacts values showing that they have a similar magnitude. This occurs in spite of the

Table 7 Environmental impacts of the life cycle of BC using ILCD 2011 Midpoint V1.06 methodology (this work, data converted from ReCiPe 2016) and from Silva et al. (2020) using ILCD 2011 Midpoint V1.05 methodology. In both cases, the functional unit is 1 kg of BC, dry basis

Impact categories	Units	This work	Silva et al. (2020)
Climate change midpoint, excl. biogenic carbon	[kg CO_2 eq.]	1.61E+01	1.31E+01
Climate change midpoint, incl. biogenic carbon	[kg CO_2 eq.]	1.60E+01	
Human toxicity midpoint, cancer effects	[CTUh][a]	1.20E-06	3.38E-07
Human toxicity midpoint, non-cancer effects	[CTUh]	1.13E-05	1.45E-05
Acidification	[mole of H^+ eq.]	6.42E-02	7.90E-02
Freshwater eutrophication	[kg P eq.]	3.99E-03	2.92E-03
Marine eutrophication	[kg N eq.]	2.41E-02	2.76E-02
Freshwater ecotoxicity	[CTUe][b]	7.81E+01	6.45E+01

[a]Comparative toxic unit for human

[b]Comparative toxic unit ecotoxicity

different assumptions, with regards to the transport of raw materials from the production site to the BC facility (included in this work but not in the study by Silva et al. (2020)) and different design production scales. The exception is verified for the human toxicity values (with cancer effects). However, although larger differences are noted in this case, the magnitude of the values is quite small; hence, the differences are not quite relevant. Overall it is possible to conclude that the impact categories present, in general, values within the same order of magnitude, in some cases quite close.

3.4 Sensitivity analysis

Table 8 illustrates the variation of the impact categories from the sensitivity analysis, taking as reference the values from Table 6.

These results show that an increase by 50% in the distance of transport (scenario 1) expectably had the lowest overall environmental impact, since the transport of raw materials already had a very low overall environmental impact (Table S5). Scenario 2 reveals an increase in "Climate change (including and excluding biogenic carbon)" and "Land use" by 10–15%. This increase affected mainly the static fermentation culture, downstream process and inoculum propagation, which were shown to consume the highest amount of energy (Fig. 3), thus impacted mainly on climate change. Regarding the other impact categories, the original values were already low, thus the increase was not significant (Table S6).

For scenario 3, the 50% increase in cooling water corresponds to a total increase in water consumption of 39% in the BC process chain. This scenario showed the highest variation in several impact categories, especially in the "Marine eutrophication"; however, as noted before, most of the original values have low absolute magnitude, except for climate change, human toxicity (non-cancer), and terrestrial ecotoxicity; from these, human toxicity (non-cancer) increased the most. The municipal wastewater treatment is responsible for most of these environmental impacts, contributing around 42% to these increases (Table S7). In ReCiPe methodology, the toxicity of chemicals to the environmental and human health is translated in five impacts categories, "Freshwater ecotoxicity", "Human toxicity, cancer", "Human toxicity, non-cancer", "Marine ecotoxicity", and "Terrestrial ecotoxicity", measured in kg 1,4-DB equivalents. As stated before, both "EU-28: Tap water from groundwater" and wastewater treatment process use several chemical in their process chain. Subsequently, in scenario 3, these two processes are responsible for the rise in several environmental impacts. Finally, the production and transport of material occur outside the BC production facility; thus, their water consumption values did not change.

Table 8 Results of the sensitivity analysis for the impact categories established in ReCiPe 2016 method. The variation relative to the original value is presented in %

Impact categories	Units	Original value	Scenario 1 (distance)	Scenario 2 (electricity)	Scenario 3 (water)
Climate change, default, excl. biogenic carbon	[kg CO_2 eq.]	1.68E+01	0.6%	10.1%	4.2%
Climate change, incl. biogenic carbon	[kg CO_2 eq.]	1.67E+01	1.2%	10.8%	7.2%
Fine particulate matter formation	[kg PM2.5 eq.]	1.57E-02	1.3%	4.5%	1.9%
Fossil depletion	[kg oil eq.]	6.57E+00	0.8%	7.5%	2.0%
Freshwater consumption	[m^3]	4.70E-01	0.0%	8.9%	-0.9%
Freshwater ecotoxicity	[kg 1,4-DB eq.][a]	8.63E-02	0.0%	0.1%	12.7%
Freshwater eutrophication	[kg P eq.]	3.98E-03	0.0%	0.3%	16.6%
Human toxicity, cancer	[kg 1,4-DB eq.]	8.26E-01	0.0%	0.2%	6.7%
Human toxicity, non-cancer	[kg 1,4-DB eq.]	1.38E+01	0.0%	0.0%	18.1%
Ionizing radiation	[Bq C-60 eq. to air]	3.42E-01	0.0%	1.5%	3.5%
Land use	[Annual crop eq.·y]	9.67E-01	0.0%	15.8%	1.7%
Marine ecotoxicity	[kg 1,4-DB eq.]	1.23E-01	0.0%	0.0%	12.2%
Marine eutrophication	[kg N eq.]	4.09E-03	0.0%	0.7%	30.8%
Metal depletion	[kg Cu eq.]	1.13E-01	0.0%	0.9%	16.8%
Photochemical ozone formation, ecosystems	[kg NOx eq.]	3.02E-02	4.0%	7.6%	3.3%
Photochemical ozone formation, human health	[kg NOx eq.]	2.88E-02	4.2%	8.0%	3.5%
Stratospheric ozone depletion	[kg CFC-11 eq.]	1.53E-05	0.7%	4.6%	19.6%
Terrestrial acidification	[kg SO2 eq.]	4.25E-02	1.2%	4.9%	2.4%
Terrestrial ecotoxicity	[kg 1,4-DB eq.]	1.56E+01	0.0%	1.9%	1.3%

[a]Expressed using the reference unit, kg 1,4-dichlorobenzene (1,4-DB) equivalent

4 Conclusion

In this work, a life cycle assessment (LCA) was used to a production process design of BC under static culture conditions, including wastewater treatment, following a cradle-to-gate approach. A considerable amount of water was consumed (36.1 ton/kg BC), mostly being treated and emitted to the environment (to fresh water). Most of the consumed water was used in secondary processes, such as the production of raw materials. This was also responsible for most of the environmental impacts, especially the production of corn syrup and sodium dihydrogen phosphate, while the culture medium preparation, inoculum propagation, static culture fermentation, and downstream process were the most environmental-friendly stage of the life cycle. In sum, the BC production factory per se had little contribution to the consumption of resources and environmental impact of the BC product life cycle. However the generated wastewater requires treatment through the municipal wastewater treatment plant, which in turn has its own environmental impacts.

A comparative analysis of the environmental impact of the BC production was also done based on literature data, using ILCD 2011 Midpoint V1.06a. The results showed a similar order of magnitude in the estimated environmental impacts, supporting the conclusion that consistent results were obtained.

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