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An approach for implementing ecodesign at early research stage: A case study of bacterial cellulose production



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ABSTRACT

Previous ecodesign approaches for the laboratory (lab) stage of product development have focused on studying one production route at a time and varying process parameters or equipment. Comparisons of alternative production routes have not been investigated, nor have been proposed specific assessments for each technology readiness level (TRL) at the lab stage of product development. This paper presents and improve an ecodesign approach for TRLs at the lab stage. This approach integrates life cycle assessment with process modeling at the industrial scale. It was applied to assess alternative bacterial cellulose (BC) production routes at the lab scale (hydrolyzed soybean molasses - HSM, diluted soybean molasses - DSM, supplemented cashew juice - SCJ, and synthetic medium Hestrin & Schramm - HS). Our results show that the HSM route exhibited better environmental performance than the other three routes. When compared to the HS route at lab scale, the HSM route reduced in 100%, in climate change, acidification and freshwater eutrophication, and in 95% the impact in marine eutrophication and freshwater ecotoxicity. Among the investigated changes in the HSM route, chemical exchange at purification phase proved possible and reduced the impact on water scarcity in 50%, at lab scale. When the HSM route was compared to the HS, at modeled industrial scale, there were no significant differences in their environmental impacts. The results from this case study allowed us to optimize the proposed ecodesign approach. We recommend that life cycle assessment (LCA) be performed at lab scale to determine the technological route with the least impact, identify critical inputs (disregarding water and energy), investigate changes that reduce environmental impacts without affecting product quality, and characterize liquid effluents. At the modeled industrial scale, the assessment of the selected route should focus on the identification of critical phases for improving water and energy efficiencies.

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1. Introduction

International efforts to achieve Sustainable Development Goals (SDGs), particularly SDG 12, which ensures sustainable

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consumption and production patterns, has required that industries invest in product ecodesign (United Nations, 2019). Ecodesign merges the evaluation of environmental performance with technological development, anticipating possible negative environmental impacts and aiming to improve the environmental performance of new production routes and products (Jeswiet and Hauschild, 2005).

Governments around the world have promoted ecodesign. One example is the European Union's Directive for Ecodesign (EU, 2009), whose purpose is to identify optimization in production routes, improve resource use efficiency, reduce pollution emissions,

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and develop new products with reduced environmental impact. Furthermore, the Responsible Research and Innovation (RRI) policy (Matthews et al., 2019) promotes the systematic integration of environmental assessment in early research.

Over the last twenty years, many approaches have been proposed for incorporating ecodesign into the research and development (R&D) processes. Earlier studies highlighted the need to consider the product life cycle to avoid changing impacts along processes in a production chain (Jeswiet and Hauschild, 2005). Most of these proposed approaches, however, applied streamlined LCA, focusing on specific life cycle stages, such as the production or end-of-life stages as well as environmental impacts (streamlined approaches) (Rossi et al., 2016; Rousseaux et al., 2017). In addition, application at late product development stages, when pilot production plants are available for gathering inventory data, were devised (Buyle et al., 2019).

Life cycle assessment (LCA), as preconized by ISO 14040 (2006), has not been applied widely owing to scant information and high uncertainties at early research stages, as well as the long time period and high expertise required (Rossi et al., 2016). To overcome these challenges, recommendations have been proposed, encompassing the need to report and account for uncertainties whenever possible, include alternative functional units, apply scenario analysis, and avoid comparisons of processes designed for different production scales (Giesen et al., 2020; Buyle et al., 2019).

Fernandez-dacosta et al. (2019) and Buyle et al. (2019) highlighted the need to develop ecodesign approaches for specific technology readiness levels (TRLs), specifically those related to product conceptualization (TRLs 1 and 2) and process definitions at the laboratory (lab) scale (TRLs 3 to 5). In these later levels, production processes are tested (TRL 3), improved (TRL 4), and prototyped or modeled for future production at pilot and industrial scales (TRL 5). These lab developments are very important because they define the product production route, its environmental impacts, and associated costs.

This set of recommendations for LCA application at early R&D (TRLs 1 to 5) has fostered the development of ecodesign approaches to support decisions at both product conceptualization (Hung et al., 2018; Blanco et al., 2020) and process definition at the lab scale (Piccinno et al., 2018a; Galli et al., 2018). For the concept level of product development (TRLs 1 and 2), Hung et al. (2018) proposed a semi-quantitative life cycle approach for selecting new materials, considering published data on their characteristics and possible inputs used to obtain them. Blanco et al. (2020) presented a probabilistic life cycle approach for considering uncertainty when comparing production pathways envisaged for new products.

Some previous studies proposed approaches for assessing and reducing environmental impacts at TRLs 3 to 5. Piccinno et al. (2016, 2018a) proposed an LCA approach for collecting inventory data at the lab scale, modeling energy use at the pilot scale, and assessing the eco-efficiency of modeled production pathways. Furthermore, Galli et al. (2018) modeled the production of oxygen-enriched air at the industrial scale, considering lab pathways with different temperature, pressure, and water flow rate. This later study compared costs and LCA results of all pathways that were up scaled, concluding that the best environmental pathway led to higher costs.

Although these previous approaches exhibited the feasibility and benefits of integrating environmental evaluation at the early lab stage, they focused on studying one production route at a time. These studies did not made comparisons of alternative production routes available at TRL 3 for further selection and improvement of a best performing route. Furthermore, they have not proposed a stepwise procedure for TRLs at the lab stage of product development. This work presents, applies, and improves an ecodesign approach for selecting and improving a production route at the lab scale. This approach presents steps for TRLs 3 to 5, integrating LCA with process modeling at the industrial scale. We applied this approach to analyze alternative bacterial cellulose (BC) production routes that produce BC with similar quality and used our results to improve the baseline approach.

The choice for studying BC was due to the challenge that R&D teams currently face to identify which BC route at the lab scale to select for future production at pilot scale. BC is obtained from microbial fermentation, with many alternative production routes currently under investigation at the lab scale (Jang et al., 2017). The production of BC at pilot scale has been a challenge pursued by R&D groups around the world because the costs related to its production in a static condition, using the synthetic medium Hestrin & Schramm (HS) are high (Gullo et al., 2017), inhibiting its wide-spread use in other sectors such as bioplastics.

In the last decade, alternative culture mediums for BC production have been investigated with good results obtained for BC yield with cost reduction. Some residues and agro-industrial co-products were pointed out as good choices for culture mediums in tropical countries, for example, effluents from alcohol distilleries (Jahan et al., 2018), sugarcane molasses (Tyagi and Suresh, 2016) and sisal juice (Lima et al., 2017). Other agricultural waste and lowvalued products such as soybean molasses and juice from cashew bagasse are currently under investigation. However, no previous studies evaluated the environmental performance of BC production routes at any scale.

Hervy et al. (2015) compared the environmental performance of two epoxy composites reinforced with nanocellulose extracted from BC at the lab scale. This study accounted for the impacts of producing BC at the lab scale but focused on comparing epoxy composites, instead of assessing the environmental impacts of BC production routes.

2. Methods

Our methods are presented in two subsections. The first subsection presents the proposed ecodesign approach for supporting decisions regarding the environmental performance of processes at TRLs 3, 4, and 5. The second subsection provides information regarding the procedure taken and assumptions made for applying the baseline approach to analyze alternative BC production routes at the lab scale.

2.1. Baseline ecodesign approach

The baseline approach included conducting technical, environmental, and production upscaling assessments at the experimental stage of the R&D process (Fig. 1). Initially, we identified production routes with acceptable yields and product quality according to literature reviews and expert interviews. We then selected the reference route determined to be the most researched by experts.

We performed LCAs on these routes as indicated by the European Commission for the environmental impact assessment of products from the technological development phase (EC, 2019).

We describe the steps of this proposed approach ahead. Step 1 regards to action taken at TRL 1, steps 2 and 3, at TRL 4, and steps 4, 5 and 6, at TRL 5.

<u>Step 1</u>. We compared the best performing alternative routes with the reference route (LCA 1). The objective was to select the production route with the best environmental performance among all routes, including the reference route, for most of the



Fig. 1. Baseline approach for the ecodesign of processes at early research stage with application at TRLs 3, 4 and 5. * TRL: Technology readiness level; LCA: life cycle assessment.

impact categories. All routes were evaluated at the lab scale, ensuring comparability.

<u>Step 2</u>. We analyzed the critical process phases of the selected route in step 1 to identify which phases most contributed to environmental impacts (LCA 2). This assessment facilitated the investigation of possible changes in inputs used in the selected route.

<u>Step 3</u>. We compared scenarios of changes (LCA 3) proposed to the selected route in step 2. Each scenario must be technically feasible at the lab scale and either maintain or improve yield. We incorporated the scenarios that led to a reduction in most of the environmental impacts into the selected route.

<u>Step 4</u>. We modeled the selected and reference routes at the industrial scale using a process design software with an industrial equipment database that allowed process upscaling. We redesigned the routes compared by replacing lab with industrial equipment at each production stage. The use of industrial equipment typically alters energy efficiency, yield, and required production time of lab processes (Hetherington et al., 2014; Piccinno et al., 2016). The modeling of production routes at the industrial scale provided the design of different production scenarios with alternative sets of equipment. We selected the

set of equipment associated with higher yield and lower capital cost to generate an inventory of inputs and emissions.

<u>Step 5</u>. We compared the environmental impacts of upscaled routes (LCA 4) to confirm the best performance of the selected route compared to the reference route.

<u>Step 6</u>. We analyzed the critical points of the selected upscaled route to determine improvement opportunities. It was possible that process phases other from those indicated in Step 2 would become relevant at the industrial production scale, as discussed by Piccinno et al. (2016). This analysis led to the identification of other critical points and new investigations aimed at improving the environmental performance of the selected route.

2.2. Case study: BC production

2.2.1. BC characterization and evaluated production routes

We applied the preliminary ecodesign approach (Fig. 1) to the choice of production routes of BC under development in the laboratories of Embrapa Tropical Agroindustry and in the Lab of Food Analysis at Londrina State University in Brazil. According to BC experts, the most widely used BC production route utilizes the synthetic medium Hestrin & Schramm (HS); thus, we selected this

reference route. The medium is a mixture of glucose, peptone, yeast extract, citric acid, and sodium phosphate (Hestrin and Schramm, 1954).

The following routes were investigated: I) hydrolyzed soybean molasses (HSM), II) diluted soybean molasses (DSM), and III) supplemented cashew juice (SCJ). These lab routes resulted in BCs with similar quality.

The parameters used to evaluate the quality of BC were thermal stability and the crystallinity index. The analytical methods applied to determine these parameters are described in the supplementary material (Appendix A.1).

A description of the compared routes is shown in Table 1. In addition to using different culture mediums, these routes differed by bacteria type, inoculum amount, supplementation, culturing time, reagent concentration, and drying conditions. The HS, SCJ, and HSM routes used standard strains from the American Type Culture Collection (*Komagataeibacter xylinus* ATCC 53582) for the origin of the bacteria. The DSM route used a strain isolated from the vinegar industry (Gomes, 2017).

For the amount of inoculum, HS and SCJ routes used 3 vol% of inoculum, while DSM and HSM routes used 10 vol%. For supplementation, only DSM and HSM routes supplemented the culture medium with ethanol (2 vol%). The static cultivation time was 10 days for HS, SCJ, and HSM routes, and the cultivation time was 14 days for DSM.

For purification, the HS, SCJ, and HSM routes used NaOH 2% and the DSM NaOH route 4%, varying the amount of repetitions of the alkaline treatment and the temperature at which they occurred. The drying temperature for these routes differed, with DSM using a higher temperature (105 $^{\circ}$ C) and lower time (8 h) than the other routes.

Table 1

Description of BC production routes (HS, SCJ, DSM e HSM) at the lab scale.

Route/phase	Hestrin & Schramm Medium (HS)	Supplemented Cashew Juice (SCJ)	Diluted Soybean Molasses (DSM)	Hydrolyzed Soybean Molasses (HSM)
Microorganism maintenance	Working cultures of <i>K. xylinus</i> (ATCC 53582) were subcultured at regular intervals of 2 weeks on slant HS agar medium and incubated at 30 °C for 2 days in a B.O.D incubator and stored at 4–6 °C until use.	Working cultures of <i>K. xylinus</i> (ATCC 53582) were routinely prepared on slant HS agar medium and incubated at 30 °C for 2 days in a B.O.D incubator and stored at 4–6 °C until use.	The bacterial species used was previously isolated from a vinegar industry and identified as Komagataeibacter sp. V-05. The organism was incubated on MYP (Mannitol Egg Yolk Polymyxin Agar) medium plates at 30 °C for 5 days in a B.O.D incubator.	Working cultures of <i>K. xylinus</i> (ATCC 53582) were subcultured at regular intervals of 2 weeks on slant HS agar medium and incubated at 30 °C for 2 days in a B.O.D incubator and stored at 4–6 °C until use.
Pre-activation	A pre-culture was prepared by transferring a cellulose pellicle from the HS working culture tube into 6 mL of HS broth medium in 15 mL falcon tubes and incubating the preculture in a B.O.D incubator without agitation at 30 °C for 3 days.	A pre-culture was prepared by transferring a cellulose pellicle from the HS working culture tube into 6 mL of HS broth medium in 15 mL falcon tubes and incubating the preculture in a B.O.D incubator without agitation at 30 °C for 3 days.	A loop of colonies was transferred into Erlenmeyer flasks with HS broth and was incubated for 10 days at 30 °C, without agitation.	A pre-culture was prepared by transferring a cellulose pellicle from the HS working culture tube into 15 mL of HS agar slant in 50 mL falcon tubes and incubating the preculture in a B.O.D incubator without agitation at 30 °C for 3 days.
Inoculum propagation	3% (v/v) of the pre-culture was aseptically transferred into 100 mL of HS medium in Schott glass bottles (250 mL) and incubated without agitation at 30 °C for 4 days in a B.O.D incubator.	3% (v/v) of the pre-culture were added into Schott glass bottles (250 mL) containing 100 mL of HS broth medium and incubated without agitation in a B.O.D incubator at 30 °C for 3 days.	A loop of colonies was transferred into Erlenmeyer flasks with HS broth and was incubated for 10 days at 30 °C, without agitation.	The pre-culture from previous step was shaken vigorously to release the attached cells from the cellulose pellicle and then the BC pellicle was aseptically transferred into 100 mL of HS medium and incubated without agitation at 30 °C for 3 days in a B.O.D incubator.
Preparation of culture medium	The Hestrin and Schramm (HS) medium was used as growth medium (20 g L ⁻¹ glucose, 5 g L ⁻¹ peptone, 5 g L ⁻¹ yeast extract, 1.15 g L ⁻¹ citric acid and 2.7 g L ⁻¹ Na ₂ HPO ₄ , pH 5).	Cashew apple juice was diluted 6-fold with distilled water and then supplemented with 5 g L^{-1} peptone and 5 g L^{-1} yeast extract, pH 5.	Diluted soybean molasses (20° Brix)	75 g of crude soybean molasses was diluted with 1L distilled water and then 5% (v/v) of 1M H_2SO_4 were added to the molasses solution, which was then heated at 90 °C for 10 min, retained until room temperature, the pH was adjusted to 6, and then the solution was vacuum filtered.
Static culture fermentation	For BC production 3% (v/v) inoculum were added into Schott glass bottles (250 mL) containing 70 mL of HS broth medium, which was then incubated without agitation (static) in B.O.D incubator at 30 °C for 10 days	For BC production 3% (v/v) inoculum were added into Schott glass bottles (250 mL) containing 70 mL of supplemented cashew apple juice, which was then incubated without agitation (static) in B.O.D incubator at 30 °C for 10 days	For BC production 10% (v/v) inoculum and 2% (v/v) soybean ethanol were added into Schott glass bottles (500 mL) containing 100 mL of soybean molasses (20° Brix) and incubated under static condition in B O D incubator at 30 °C for 14 days	For BC production 10% (v/v) inoculum and 2% (v/v) ethanol were added into Schott glass bottles (250 mL) containing 50 mL of hydrolyzed soybean molasses and incubated under static condition in B O D incubator at 30 °C for 10 days
Purification	The produced BC was collected, rinsed in water and then boiled twice in water at 100 °C for 1h, followed by immersion in NaOH 2% (v/v) at 80 °C for 90 min to remove medium components, attached cells and other residues.	The produced BC was purified by at least five immersions in NaOH 2% (v/ v) at 80 °C for 1h, until the complete removal of medium components, attached cells and other residues.	The produced BC was collected, rinsed in water and then in NaOH 4% (w/v) at 80 °C for 30 min to remove medium components, attached cells and other residues.	The produced BC was collected, rinsed in water and then boiled twice in water at 90 °C for 40 min, followed by immersion in NaOH 2% (v/v) at 90 °C for 1h to remove medium components, attached cells and other residues.
Neutralization	The BC was washed in distilled water	The BC was washed in distilled water	The BC was washed in distilled water	The BC was washed in distilled water
Drying	The purified cellulose was dried at 50 °C for 24h to constant weight and the mass was determined.	The purified cellulose was dried at 50 °C for 24h to constant weight and the mass was determined.	The purified cellulose was dried at 105 °C for 8h.	The purified cellulose was dried at $50 \circ C$ for 24h to constant weight and the mass was determined.

2.2.2. LCA: purpose and scope

At LCA 1 (Fig. 1), the alternative routes HSM, DSM, and SCJ were compared with the HS route. In LCA 4, the route with the lowest impact (selected in LCA 1) was compared with the HS reference route and both were modeled at the industrial scale. LCAs 2 and 5 aimed to identify critical process phases at lab and modeled industrial scales. LCAs 3 and 6 compared scenarios at lab and modeled industrial scales.

All proposed LCAs (1–6) were from cradle to gate, including the stages of raw material extraction, input production, and BC production. The transportation of inputs to the BC production unit and the use and end-of-life stages were disregarded. At this level of technological development, there are no studies showing possible distances between the input industries and the BC facility that was considered as a location at the Brazilian Midwest region (see Section 2.2.2.2).

The functional unit used in LCAs at the lab scale (LCAs 1, 2, and 3 in Fig. 1) was the production of 1 g of BC in 35 days. In the LCAs at the modeled industrial scale (LCAs 4, 5, and 6) (Fig. 1), the functional unit was the production of 1 t of BC (80% moisture), considering 1 year of production. These functional units were defined to better represent the production function occurring at each production scale. Lab production per batch is measured in grams with process time around a month, while industrial production, per ton with production occurring over the year.

For the difference in the duration of the four routes compared in LCA 1, the evaluation was standardized for a production time of 35 days (time of the route of greatest duration). The yield of each route was then recalculated for 35 days, and the mass balance was performed for this new duration time.

In all LCAs performed, the following phases of BC production were considered: microorganism maintenance, preactivation, inoculum propagation, culture medium preparation, static cultivation, purification, neutralization, pellicle drying, and effluent treatment (Fig. 2). At the industrial scale, the phases of microorganism maintenance, preactivation and inoculum propagation were considered aggregated. In addition, the drying phase was substituted by centrifugation and sterilization because the industrial plant was modeled to produce BC for use as skin dressing, presenting 80% of humidity. Packaging in laminated plastic was also added at modeled industrial scale.

2.2.3. LCA: inventory data

Primary inventory data for BC production was collected at the laboratories of Embrapa and Londrina State University (foreground process). The scale up modeling was based on this data. Inventories for the production of inputs (background processes), used in BC production, were from secondary databases and the literature.

2.2.3.1. BC production at the lab scale. In LCAs 1, 2, and 3, the quantification of inputs, effluents, and yield at HS, SCJ, DSM, and HSM routes was performed between 2017 and 2018. Three trials were performed for each route.

For energy, consumption was calculated according to the type of lab equipment used at each BC production phase. In the microorganism maintenance, preactivation, inoculum propagation, and static culture fermentation phases, the energy required by the biologic oxygen demand incubator and the biological safety cabinet was calculated with Equation (1). This equation represents the energy consumption of equipment whose primary source of energy supply is electrical. In addition, a term called capacity factor was adopted in this equation. This term represents the volumetric capacity truly occupied by the equipment over the maximum volumetric capacity. For example, the BOD incubator has a nominal power of 1000 W to support a maximum volumetric capacity of up to 334 L; however, the process required only 1 L and therefore, the power required to supply this amount of volume used is proportionally less than the nominal (1000 W). The power and maximum load capacity of the equipment were derived from equipment manuals and catalogs published by the respective manufacturers.

$$E = P * t * (C_{used} / C_{max})$$
(Equation 1)

where *E* is the energy consumption (kWh); *P* is the power required by the equipment (kW); *t* is the amount of time that the equipment was used (h), and *Cused/Cmax* is the relation between the capacity used and the maximum load capacity of the equipment (L).

The sterilization process was performed with an autoclave that uses moist heat under pressure; hence, the amount of energy required for sterilization was calculated considering the sensible heat (Equation (2)) and the convective heat transfer (Equation (3)). Two forms of heat propagation generate the energy required to perform sterilization in an autoclave. The first form requires heating the water in its liquid state until it is vaporized. This phenomenon is represented in Equation (2). The second form is the convection caused by the movement of convective currents, represented in Equation (3). Equation (3) was also used to calculate the energy required by the drying oven used to dry the BC pellicles.

$$Qs = m^* Cp^* \Delta T$$
 (Equation 2)

where Q_s is the sensible heat (kWh); *m* is the mass (kg); *Cp* is the calorific capacity (J/kg.K), and ΔT is the temperature difference (K).

$$Qh = h^*A^* \Delta T$$
 (Equation 3)

where Qh is the heat flux (kWh); h is the convective heat transfer coefficient (W/m².K); A is the surface area where the heat transfer takes place (m²), and ΔT is the temperature difference (K).

In the culture medium preparation phase, Equations (2) and (4) were used to calculate the heating energy used during the acid hydrolysis in the HSM route. Equation (4) represents the energy transferred by the metal surface of the heating plate.

$$Qu = U^*A^*\Delta T$$
 (Equation 4)

where Qu is the conductive heat transfer (kW.h); U is the overall heat transfer coefficient (W/m². K); A is the heat transfer area of the surface (m²), and ΔT is the variation of temperature (K).

The inventories of BC production at the lab scale can be found in Tables SM1 to SM4 in the supplementary material. For an example about how to make energy calculation see Appendix A.2 in the supplementary material.

2.2.3.2. BC production at modeled industrial scale. In LCAs 4, 5, and 6, the selected route (with the lowest environmental impact identified in LCA 1) and the reference route (HS) were modeled using SuperPro Designer® software, version 10 (Intelligen Inc., New Jersey, US). For the modeling, data regarding the fermentation conditions to produce BC at the lab scale, related to temperature and reaction time, the quantity of materials inputs in each step, and production of BC per liter of culture medium, were utilized.

Different sets of equipment for producing BC were compared for choosing the set that led to the best relation between yield and capital costs. The capital costs for the BC routes modeled at the industrial scale were calculated using SuperPro database. This database had data regarding suppliers and machineries with different processing capacity.

The conceptual design for the BC industrial-scale plant modeled by Dourado et al. (2016) served as the basis for choosing the



Fig. 2. BC product system in HS, SCJ, DSM and MSH routes.

* CM HS means cultivation media Hestrin & Schramm; CM HSM, cultivation media Hydrolyzed Soybean Molasses; CM DSM, cultivation media Diluted Soybean Molasses; and CM SCJ, cultivation media Supplemented Cashew Juice.

production capacity of the BC industrial plant modeled in this study. This plant was considered to be located at the Brazilian Midwest region, close to a main soybean oil company that produces soybean molasses. It has an average annual production size of 430 t of BC with 80% humidity, in a batch regime, corresponding to monthly processing of 60 t of culture medium, five batches/month. The list of equipment used in each production phase at lab and industrial scales can be found in the supplementary material (Table SM5). No heat-integration or water reuse facilities were considered in this study but are currently under research.

The mass and energy balance provided in the simulation report of SuperPro was used to determine the BC production inventory at the industrial scale. The inventories of routes modeled at the industrial scale can be found in the supplementary material (Tables SM6 and SM7).

2.2.3.3. LCA: secondary inventory data. The following inventories were obtained from published literature:

- 1) Cashew production (Figueirêdo et al., 2016) and supplemented cashew juice (Pinheiro, 2016).
- 2) Chemical, energy, effluent treatment, and sugar production and soybean crop production (ecoinvent v.3.0; Weidema et al., 2013). This database has inventories for a high variety of processes in all sectors and the following inventories for Brazil used in this study: electricity, sugar and sugarcane production. This study considered the average Brazilian electricity mix from 2008 to 2014, which included the transformation from high to

medium voltage and electricity transmission. The Brazilian mix was used because the BC production at the lab scale occurred in Brazilian labs and the one modeled at the industrial scale, was located in the Midwest region (see Section 2.2.3.2). For effluent treatment, the electrical energy and water emissions inputs were replaced for the Brazilian context, attributing a higher degree of uncertainty to these data. For sugar, the composition of HS medium consisted of 20.0 g L⁻¹ glucose, 5.0 g L⁻¹ peptone, 5.0 g L⁻¹ yeast extract, 1.5 g L⁻¹ citric acid, and 2.7 g L⁻¹ sodium phosphate (Hestrin and Schramm, 1954). The glucose production data were not available in the SimaPro® software version 9.0.0.35 databases (PRé Consultants, 2019); therefore, a similar proxy route produced table sugar from sugarcane. It is composed of approximately 99% sucrose.

3) Soybean molasses (Agri-footprint v. 1.0; Blonk Agri-footprint BV, 2014). The inventory of soybean molasses and soybean crop production were not available in the ecoinvent database and were taken from Agri-footprint. Soybean molasses is a co-product of the evaporation of liquids during the drying of soybean protein concentrate.

The correspondence between routes and names of inventories from ecoinvent and Agri-footprint is described in the supplementary material (Table SM8).

Some inventories taken from the ecoinvent and Agri-footprint databases applied allocation when coproducts were produced (e.g. soybean molasses and oil). This study used inventories with mass allocation in both databases. For ecoinvent inventories, the "point-of-substitution (APOS)" partitioning was used. This allocation procedure considers products and coproducts from waste treatment in a combined system of activities (Weidema et al., 2013).

2.2.4. LCA: impact assessment

The following methods were applied for assessing the environmental impacts on LCAs 1 and 2: I) ILCD 2011 Midpoint V1.05, for climate change, soil acidification, freshwater ecotoxicity, human toxicity, cancer and non-cancer effects, marine and freshwater eutrophication, and II) AWARE V1.00 for water scarcity. The ILCD 2011 Midpoint V1.05 was chosen because it resulted from a broad scientific consensus (JRC and IES, 2011), while Aware was indicated for use in Brazil (Castro et al., 2018). SimaPro® software version 9.0.0.35 was used to perform the impact assessment (PRé Consultants, 2019).

2.2.5. LCA: uncertainty analysis

We used the Monte Carlo method to conduct the uncertainty analysis for comparisons between BC production routes. The inventory data was considered to have a lognormal distribution. This probability distribution was adopted because life populations usually present this type of distribution and it is used in most of the ecoinvent inventories (Weidema et al., 2013).

The standard deviation of all inventory data was calculated using the Pedigree matrix (Weidema and Wesnaes, 1996). For LCA 1, the overall uncertainty attributed to inventory parameters of the studied routes was very low (1) because input and outputs were directly measured at the lab.

Inventory data for LCA 2 were obtained from mass and energy balances performed in the software Superpro. Higher uncertainty scores were attributed for each criteria of the Pedigree Matrix. 1) A score of 3 for reliability because data from the simulation of modeled plants at the industrial scale were used. II) A score of 4 for completeness because equipment efficiency and energy requirements in the SuperPro database were obtained from manufacturers in the United States of America (USA). III) A score of 1 for temporal correlation because data were gathered over less than three years. IV) A score of 4 for geographic correlation because the industrial plant was thought to be in Brazil, but the equipment in this plant was produced in the USA. V) A score of 3 for technological correlation because data were derived from the processes and materials under study using different technology.

When comparing two routes (A and B), we calculated how many times route A presented a lower environmental impact than route B, evaluating the impact of A - B < 0 in 1000 simulations (Goedkoop et al., 2016). If A - B < 0 in at least 95% of the simulations, we concluded that A caused significantly less impact than B.

3. Results

3.1. Analysis of the technical feasibility OF BC production routes

All the pre-selected routes (HSM, DSM, and SCJ) were technically feasible because they produced BC in a quantity and quality similar to that of the HS route (Table 2). The cellulose produced in these media had a crystallinity index between 69% and 85%, which is within the standard range reported in the literature (Trovatti et al., 2011; Tsouko et al., 2015) and indicates high mechanical strength. The BCs presented typical thermal behavior of BC, and the BC produced in SCJ presented a higher initial degradation temperature, indicating greater stability at high temperatures (De Salvi et al., 2014).

3.2. Environmental assessments at the lab scale

3.2.1. Comparison of production routes at the lab scale (LCA 1)

When comparing BC production routes, HSM was the least impactful route for most of the impact categories assessed, while SCJ was the most impactful (Fig. 3). The greatest impact of the SCJ route was primarily due to the lower yield of BC obtained per volume of medium used (4.6 g L^{-1}). HSM yield (11.7 g L^{-1}) was higher than the others were.

The uncertainty analysis between the best (HSM) and worst (SCJ) routes revealed that the difference between them was significant for most of the assessed impact categories (Table 3). The difference between HSM and HS was also significant for most of the categories analyzed (supplementary material, Table SM9).

HSM performed better than DSM for half of the impact categories (supplementary material, Table SM10). This occurred primarily because the mass of soybean molasses used in HSM (10.7 g/g of BC, supplementary material Table SM2) was less than half of the mass used in DSM (27.9 g/g of BC) (supplementary material Table SM4).

3.2.2. Analysis of critical points at the lab scale (LCA 2)

The contribution analysis of HSM phases (best performing route) showed that the culture medium preparation phase contributed most to the environmental impacts (Fig. 4a). The purification and inoculum propagation phases had similar environmental impacts in most categories. The input contribution analysis



Freshwater eutrophication

Fig. 3. Comparative environmental impact assessment of BC produced in HS, SCJ, DSM and MSH routes at the lab scale (LCA 1).

* HS is Hestrin & Schramm; HSM, Hydrolyzed Soybean Molasses; DSM, Diluted Soybean Molasses; and SCJ, Supplemented Cashew Juice.

Table 2

Characteristics of production and quality of bacterial cellulose production routes (HS, HSM, DSM and SCAJ).

Route	Production (g/L of culture medium)	Crystallinity Index (%)	initial degradation temperature (°C)
HS	8.79	85.2	312.2
HSM	11.70	75	312
DSM	9.97	69	299.3
SCAJ	4.66	80.71	318

Table 3

Uncertainty analysis in the comparison of HSM and SCJ routes at the lab scale (LCA 1), considering the production of 1g of BC.

Impact categories	Unit	SCJ	HSM	HSM < SCJ
Climate change	kg CO2 eq	7.74E-01	3.54E-01	100%
Human toxicity, cancer effects	CTUh	2.33E-08	8.63E-09	83%
Human toxicity, non-cancer effects	CTUh	1.79E-07	1.09E-07	50%
Acidification	molc H+ eq	3.23E-03	1.52E-03	100%
Freshwater eutrophication	kg P eq	2.00E-04	7.36E-05	100%
Marine eutrophication	kg N eq	6.70E-04	3.60E-04	100%
Freshwater ecotoxicity	CTUe	6.81E+00	2.87E+00	94%
Water scarcity	m3	4.47E+02	9.54E-01	100%



Fig. 4. Contribution of process phases and inputs in route HSM for the environmental impacts of BC at the lab scale (LCA 2).

a) Environmental impacts of route HSM per production phase, at the lab scale.

b) Environmental impacts of route HSM per input, at the lab scale.

showed that electricity was the main source of impacts at almost all phases (Fig. 4b).

In the Brazilian electricity mix, 75.6% of the average internal electricity supply came from hydroelectric plants (ANEEL, 2008). Although this is considered a renewable energy source, its production requires an infrastructure that promotes deforestation, flooding of large areas (often forest), decomposition of organic matter, emission of greenhouse gases, nutrient enrichment (decomposition of organic matter), and water consumption (evaporation of water) in reservoirs.

In addition to energy consumption, the impacts were primarily

the results of soybean molasses (coproduct of soybean oil production), especially for human toxicity, non-cancer effects (38%) and marine eutrophication (23%) (Fig. 4b). Soybean crop production contributed from 38 to 99% of the soybean molasses impacts, according to the category analyzed (supplementary material, Fig. SM3). Impacts due to ethanol and sodium hydroxide (NaOH) were also identified with a greater contribution to water scarcity.

3.2.3. Scenario analysis at the lab scale (LCA 3)

Possibilities for changes in the critical processes were assessed based on the critical analysis (LCA 2). It was known that energy consumption would change when moving from the lab to industrial production and so, modifications in the other relevant inputs (soybean molasses, ethanol, and NaOH) were prioritized and investigated.

The amount of soybean molasses and the time and temperature binomial had already been optimized in the lab and thus, there was no possibility of reducing this input in the preparation phase of the culture medium.

The experiments conducted to evaluate the possibility of reducing or eliminating ethanol in the production of BC revealed that without the addition of ethanol, BC production fell by 54%. The use of ethanol improved the synthesis of BC because ethanol acted as a carbon source in the early phase of fermentation (Li et al., 2012). This input could, therefore, not be reduced.

Finally, two scenarios for the possibility of changing NaOH for the purification phase were investigated in the lab: (1) replacing 2 vol% NaOH with KOH in the same concentration and quantity and (2) reducing the amount of alkali washing in the purification phase by inserting 1 vol% H_2O_2 in the first wash with 2 vol% NaOH. The yield did not change in these scenarios and LCA 3 was performed.

Scenario 1 led to a 50% decrease on water scarcity without significantly changing the other impacts. Scenario 2 did not lead to a change in impacts (supplementary material, Fig. SM5); thus, the reagent change proposed in Scenario 1 was implemented in the HSM route.

3.3. Environmental assessments at the industrial scale

3.3.1. Comparison of BC routes modeled at the industrial scale (LCA 4)

The BC production modeled at the industrial scale had a yield of 35 t/month at HSM and 26 t/month at HS (reference route). The comparison of these routes after process upscaling showed that HSM and HS had similar impacts, considering a confidence index of 95% in the uncertainty analysis (Fig. 5). Although the HSM route required a greater variety of inputs (e.g., sulfuric acid, sodium hydroxide, and ethanol), the impacts of this route were offset by its

increased yield (426 t/year). The yield on the HS route was 318 t/ year.

3.3.2. Critical point analysis at modeled industrial scale (LCA 5)

When analyzing the contribution of process phases in the HSM route, the culture medium preparation was the most relevant phase in all impact categories (Fig. 6a). When evaluating the contribution of inputs, there was a large reduction in energy consumption (Fig. 6b), highlighting the importance of other inputs (soybean molasses, ethanol, and NaOH).

A consultation with BC experts revealed no more possibilities for equipment changing at both HSM and HS routes, and because new scenarios were not proposed, LCA 6 (Fig. 1) was not performed. The next step in this work will be to design a water recirculating and heat-integration system.

4. Discussion

Upon analyzing the results, three questions emerged: What are the main changes occurring when moving from lab-scale to industrial-scale? Can the baseline ecodesign approach be improved? What are the primary uncertainties of the proposed approach and case study?

4.1. Main changes occurring when production is scaled up

Upscaling BC production changed water and energy consumption, as well as the magnitude of impacts across all assessed categories of HSM and HS routes. In addition, it changed the identification of critical production phases. The most impactful inputs were maintained at both scales of evaluation, when energy was disregarded.

A high total energy reduction occurred in both HSM and HS (99% in Table 4). This reduction occurred despite an increase in energy consumption in many activities manually performed in the lab, such as mixing of materials and their transportation among lab devices and packaging. Industrial equipment is much more energy



Fig. 5. Uncertainty analysis of the comparison of HS and HSM environmental impacts of BC at the industrial scale (LCA 4).

* HSM \geq HS shows the percentage of times that the impact of HSM route was higher or equal to the impact of the HS route (HSM – HS \geq 0), in 1000 Monte Carlo simulations. Values higher or equal to 95% are considered significant.

HSM < HS shows the percentage of times that the impact of HSM route was lower than the impact of the HS route (HSM - HS < 0), in 1000 Monte Carlo simulations. Values higher or equal to 95% are considered significant.





Fig. 6. Contribution of process phases and inputs in route HSM for the environmental impacts of BC at the industrial scale (LCA 5). a) Environmental impacts of route HSM per production phase, modeled at the industrial scale. b) Environmental impacts of route HSM per input, modeled at the industrial scale.

Table 4

Energy demand in each phase of HSM and HS routes, considering the production of 1 g of BC/month, at the lab and industrial scales.

Production phases	HSM			HS		
	Lab-scale (kWh)	Industry scale (kWh)	Increase or decrease (%)	Lab-scale (kWh)	Industry scale (kWh)	Increase or decrease (%)
Microorganism maintenance	1.40E-01			1.86E-01		
Preactivation	1.60E-01	2.05E-08	100%	2.13E-01	2.82E-08	100%
Inoculum propagation	2.63E-01			3.50E-01		
Preparation of culture medium	2.47E-01	1.85E-04	99.93%	3.30E-01	7.57E-05	99.98%
Static culture fermentation	5.49E-02	3.04E-05	99.94%	7.32E-02	2.90E-05	99.96%
Purification	2.94E-01	1.18E-07	100%	3.91E-01	1.25E-07	100%
Neutralization	0.00E + 00	1.18E-07	100% ^a	0.00E+00	1.25E-07	100% ^a
Drying	7.71E-02	4.41E-05	99.94%	1.03E-01	6.57E-05	99.94%
Packaging	0.00E + 00	1.66E-07	100% ^a	0.00E+00	2.29E-07	100% ^a
Total	1.24E+00	2.60E-04	99.98%	1.65E+00	1.71E-04	99.99%

^a Increase.

efficient per kg of product than lab equipment (in MSH, 1.24 kWh/ g.month at the lab and 0.0003 kWh/g.month at the industrial scale, Table 4). Another aspect is the use of steam for thermic energy in mixing tank, pasteurizer, and steam sterilizer at the industrial scale, instead of electricity, at the lab scale.

The production phases that consumed more energy changed with upscaling (Table 4). At the lab scale, the purification phase accounted for 24% of the total energy consumption in both routes. At the industrial scale, the culture media preparation answered for 44% of the consumption in HS and 71% in HSM. The reactor used to hydrolyze soybean molasses was responsible for this energy consumption in the HSM route at the industrial scale.

Reduction in total energy consumption when upscaling production was also observed by Hetherington et al. (2014), Piccinno et al. (2016), Tan et al. (2018), and Bartolozzi et al. (2019). Hetherington et al. (2014) compared food-quality oil production using lab data for modeling an industrial plant and noted a large reduction in energy consumption. This was due to a shift from batch production in the lab to a continuous process in an industrial plant. When establishing rules for the scheduling of chemical processes, Piccinno et al. (2016) observed that greater production of these products at the pilot scale promoted the reduction of energy consumption as the production capacity of the industrial plant increased. Tan et al. (2018) and Bartolozzi et al. (2019) verified a reduction in energy consumption in the LCAs of nanoproducts when moving from lab to pilot scales.

Regarding water use, there was also a high decrease in the total volume used in both HSM and HS (99%) when process phases were scaled up (Table 5). This decrease in water use is primarily attributed to the higher control and efficiency of water use at the industrial scale. The water use in the neutralization and purification phases at the lab scale were performed manually while at the industrial scale, in washer equipment.

The production phases that consumed more water in BC production changed little with the scale up (Table 5). At the lab scale, the purification and neutralization phases used more water in both routes, while at the industrial scale, the purification phase distinguished more than the other phases.

The magnitude of impacts related to the production of 1 kg of BC/month decreased considerably (at least 97%) with the change of scale in both routes and all impact categories (Table 6). Piccinno et al. (2018b), when evaluated the production of nanocellulose, Tan et al. (2018), when they studied cellulose nanocrystal foam, and Bartolozzi et al. (2019), when evaluating nanosponges of cellulose, also observed significant reductions in the values of impacts when comparing the production at the lab and at the pilot or industrial scales.

The environmental impact analysis of BC in the HSM route showed that when electricity is disregarded, the inputs that caused more impacts at the lab scale were also those at the industrial scale (Fig. 4b and Fig. SM4, supplementary material). Thus, it is necessary to disregard the electricity demand to identify critical phases and inputs that are relevant, independently of scale.

4.2. Uncertainties in the proposed approach and case study

Qualitative and quantitative uncertainties were present in this study and are discussed in this section. The qualitative uncertainty was related to the proposed ecodesign approach and is discussed considering the diamond tool proposed by Gavankar et al. (2014). The quantitative uncertainty was related to parameter uncertainty, measured applying Monte Carlo simulation to comparisons performed in steps 1, 3, 4 and 6 of the proposed approach (Fig. 1).

Gavankar et al. (2014) presented a diamond tool for researchers to communicate uncertainties of proposed models. This tool encompasses four types of uncertainties due to: I) information gaps for gathering input data; II) variability of input data, III) lack of information for performing scenario analysis; and IV) changes in the external context (technology, socioeconomic, and political).

When applying this tool to the proposed ecodesign approach, we found that it presents medium uncertainties overall. For information gaps, the uncertainty was low because the data required for comparing alternative production routes and for process scale up were available at the end of TRL 3.

The proposed approach considered the variability of input data when comparing alternative production routes. Variability in input data was considered low at lab and industrial scales because the amount of input is measured at the lab scale and linearly scaled up for modeled industrial scale.

The scenario analysis was proposed in steps 3 and 6 of the ecodesign approach. It was performed according to the knowledge of the expert team who checked the consequences of changes in yield, using the available equipment in the lab. Since equipment devices available are always limited in research labs, there will always be process constraints inhibiting the proposition and testing of possible alternatives. Therefore, this type of uncertainty was considered high.

Technology and economy are constantly changing all over the world, increasing the uncertainty in the external context. Globalization makes a change occurring in one region to affect the whole world. Furthermore, new technologies may emerge in the future, thereby reducing the costs of equipment devices and the use of resources. Thus, uncertainty in the external context was scored high for the proposed approach and is probably high for former approaches developed to support decisions at the lab scale.

For quantitative uncertainty in the case study, parameter uncertainty was present in the inventory of glucose (used in HS culture medium) and in the inventories from secondary sources. All

Table 5

Water demand in each phase of HSM and HS routes, considering the production of 1 g of BC/month, at the lab and industrial scales.

Production phases	HSM			HS		
	Lab-scale (L)	Industry scale (L)	Reduction (%)	Lab-scale (L)	Industry scale (L)	Reduction (%)
Microorganism maintenance Preactivation	2.86E-04 1.00E-03	0.00E+00	100%	4.76E-04 1.33E-03	0.00E+00	100%
Inoculum propagation Preparation of culture medium	3.43E-03 2 59F-01	477F-03	98 15%	8.19E-03 1 23F-01	2 48F-03	97 98%
Static culture fermentation	0.00E+00		100%	0.00E+00	0.00E+00	0.00%
Purification Neutralization	2.57E+00 2.57E+00	8.15E-04 3.35E-02	99.97% 98.70%	4.79E+00 2.95E+00	8.31E-04 3.82E-02	99.98% 98.70%
Drying	0.00E+00	0.00E+00	0.00%	0.00E+00	0.00E+00	0.00%
Packaging Heat transfer and CIP Total	0.00E+00 0.00E+00 5.41E+00	0.00E+00 1.51E-02 5.42E-02	0.00% 100% 99.00%	0.00E+00 0.00E+00 7.86E+00	0.00E+00 1.30E-02 5.24E-02	0.00% 100% 99.31%

Table 6	

Environmental impacts of HSM and HS routes,	considering the production of 1	l g of BC/month, at the lab a	and industrial scales.
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Impact categories	Unit	HSM		HS			
		Lab-scale	Industry scale	Reduction	Lab-scale	Industry scale	Reduction
Climate change	(kg CO2 eq)	3.52E-01	2.62E-03	99.26%	8.93E-01	1.11E-03	99.88%
Human toxicity, cancer effects	(CTUh)	8.88E-09	6.76E-11	99.24%	2.43E-08	1.07E-10	99.56%
Human toxicity, non-cancer effects	(CTUh)	1.13E-07	2.89E-09	97.43%	1.78E-07	6.53E-10	99.63%
Acidification	(molc H+ eq)	1.52E-03	1.58E-05	98.96%	3.84E-03	9.37E-06	99.76%
Freshwater eutrophication	(kg P eq)	7.49E-05	5.83E-07	99.22%	1.94E-04	4.99E-07	99.74%
Marine eutrophication	(kg N eq)	3.62E-04	5.51E-06	98.48%	8.04E-04	2.54E-06	99.68%
Freshwater ecotoxicity	(CTUe)	2.97E+00	1.29E-02	99.56%	7.85E+00	1.23E-02	99.84%
Water scarcity	(m3)	4.75E-01	1.59E-02	96.66%	9.22E-01	2.27E-02	97.54%

flows related to the production of glucose used in the HS culture medium were disregarded, being the flows of sugar table production considered instead. However, glucose is obtained from sugar and to add the glucose production flows only increases the impacts of HS route. This route uses a greater amount of HS culture medium than HSM route, making it less favorable than HSM route in LCA 1.

The inventories from secondary sources were built from best available information provided by industry and published literature, following specific quality criteria (Weidema et al., 2013; Blonk Agri-footprint BV, 2015). However, each database adopted specific quality criteria. Besides, information about exact substances were not always available and proxy data may have been used. When this latter case happened, a higher uncertainty score was applied in the Pedigree Matrix.

Besides parameter uncertainty, there was uncertainty regarding BC yield at the industrial scale because yield may be affected by production upscaling. According to Dourado et al. (2016), BC cells have high sensitivity to changes in temperature and volume of culture medium. Thus, mutation of cellulose-producing bacteria may occur in industrial plants affecting BC yield. This mutation may happen independent of the used culture medium, being of equal probability of occurrence in HSM and HS routes. This uncertainty was not considered in the parameter analysis performed with Monte Carlo and proposed in steps 3 and 6 of the proposed approach.

4.3. Analysis of the proposed ecodesign approach

Positive aspects and opportunities for improvements emerged after analyzing the proposed ecodesign approach (Fig. 1). A positive aspect of this approach was the selection of a lab-scale technological route. This action substantially reduced the resources required in the next steps related to the study of changes in critical points and industrial-scale modeling. It is important to highlight that for the initial comparison between routes to be valid, route inventories need to be all at the same production scale, as suggested by Hetherington et al. (2014).

In addition, inventories should cover all inputs required for each route, including energy and water. The BC routes that performed better at the lab scale also performed well at the modeled industrial scale. This occurred due to the maintenance of time and temperature conditions of the reactions and the types of raw material used when modeling routes at the industrial scale.

Another positive aspect was the analysis of the contribution of inputs in the LCA first performed (LCA 1) (Fig. 1). This analysis allowed for the implementation of changes in the process before greater efforts were made to model it at the industrial scale.

Modeling the lab process at the industrial scale resulted in changes regarding energy and water use, yield, and impact values. Changes in energy, yield and impacts were expected due to changes in equipment and previous evaluations findings (Piccinno et al., 2018b; Tan et al., 2018; Bartolozzi et al., 2019). For water, we expected the relation between volume and mass of BC to increase because CIP and heat steam were introduced at the industrial scale. However, this relation was drastically reduced with the scaling up primarily because of the automation of washing activities, usually performed manually at the lab scale.

Despite this reduction in water volume per kg of product, the total water volume increases with the scale up of production, being important to design appropriate effluent treatment and water reuse systems at industrial scale. However, this design of appropriate water and wastewater facilities requires physical-chemical characterization of effluents at the lab scale. This research activity should thus be considered at TRL 4 as part of the ecodesign approach of biomaterials in general, including BC.

Considering all these aspects regarding production upscaling (LCA 4), we propose the following improvements in the approach originally proposed (Fig. 7): I) in LCA 2, TRL 4, the objective should be to identify critical inputs, disregarding water and energy use because they radically changed with upscaling; II) in LCA 3, at TRL4, it is necessary to analyze the physical-chemical characteristic of liquid effluents of the selected route to allow the design of appropriate water recirculation and reuse systems as well as wastewater system; and III) in LCA 4, at TRL 5, the focus should be to identify critical phases for energy and water use in the modeled selected route, investigating opportunities for improvements in water and energy efficiencies.

5. Conclusion

This work presents an ecodesign approach for use at early R&D stages sfor selecting and improving production routes at the lab scale. We successfully applied this approach based on the integration of LCA and process modeling at the industrial scale and assessed alternative BC production routes to improve a selected route.

We observed with the BC case study that HSM was the best environmentally performing route. The analysis of the different LCAs performed allowed us to conclude that upscaling considerably reduces energy and water use as well as the magnitude of environmental impacts. Moreover, we noted that the inputs with the most impact were the same at both scales of evaluation when energy and water use were disregarded.

The analysis of results from the BC case study allowed us to optimize the proposed ecodesign approach. We recommend that LCA be performed firstly at the lab scale to select the technological route with less impact, identify critical inputs (disregarding water and energy), investigate changes that reduce environmental impacts without affecting product quality, and to characterize effluents of selected route. The improved selected route shall then be scaled up and assessed to identify critical phases and opportunities for fostering water and energy efficiencies.



Fig. 7. Improved approach for the ecodesign of processes at early research stage with application at TRLs 3, 4 and 5.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Renata de Araújo e Silva: Data curation, Writing - original draft, Investigation. Ana Iraidy Santa Brígida: Investigation, Supervision. Morsyleide de Freitas Rosa: Investigation, Project administration. Raimundo Marcelino da Silva Neto: Investigation, Software, Validation. Wilma Aparecida Spinosa: Investigation, Resources. Ednaldo Benício de Sá Filho: Software, Validation, Data curation. Maria Cléa Brito de Figueirêdo: Conceptualization, Methodology, Supervision, Writing - review & editing.

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Appendix A. Supplementary data

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