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Analysis of the Environmental Life Cycle of Bacterial Cellulose Production

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Cellulose is one of the most abundant natural polymers on earth and is mainly produced by plants, although many bacteria, especially those belonging to the genus *Gluconacetobacter*, produce a very peculiar form of cellulose with mechanical and structural properties that can be exploited in numerous industrial applications. Studies about the kinetic of microorganism's growth, helps the researchers understand more about how to obtain bacterial cellulose (BC) films, within specific measurement based on fermentation process. On the last two decades, the public and scientific interest has increased regarding the use and evolution of biodegradable plastics. Beside the fact that biodegradable plastics have the desired chemistry and physics properties that most of the synthetic plastics own, it can be obtained from renewable sources. The agroindustry residue Corn Steep Liquor (CSL) was used to replace the yeast extract and peptone as carbon and nitrogen sources aiming to minimize the cost, environmental impact and to add value to BC production. The culture medium that uses CSL to produce BC according to the life cycle analysis (LCA) showed a considerable reduction of environmental impacts. Considering a production for industrial application of the biomaterial, the reduction of production costs can also be high.
Keywords: Bacterial Cellulose, *Gluconacetobacter hansenii*; Life Cycle Analysis, Environmental impacts

1. Introduction

The biodegradable plastic or biopolymer is a polymeric material that could be able to be metabolized by organisms found in the environment, at least in one of its degradation step process Rrhim et al., 2013). BC is a type of natural biopolymer that can be synthetized by several organisms. It has a nanofibrilar network in a 3D arrangement that in its hydrated form, it can retain 99% of the water, and it has high resistance to traction, and it may be synthetized by several organisms such as bacteria, molds and yeasts (Wu et al; 2014). Based on current research, it is the beginning of a new era of industrial application of nanocellulose and nanocomposites made of BC, keep in mind that the nanocellulose has the mechanical properties, flexibility, biocompatibility, availability and low cost.
BC microfibrils are approximately 100-fold smaller than the fibrils of vegetal cellulose (Chawla et al., 2009; Gayathry; Gopalaswamy, 2014). The BC fibrous network is formed by three nanofibers, well arranged, that results on formation of a hydrogel leaf containing a high superficial area and porosity (Esa et al. 2014).
The BC production happens by fermentation process, the Hestrin-Schramm (HS), described in 1954, is the culture method used for the cultivation method. This culture approach presents a high cost, of about 30 % of the total production cost, due to its need of glycose supplementation, yeast extract and peptone for the

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fermentation process (Costa et al., 2017). Besides that, some parameters such as pH, temperature and aeration, agitation and growing time affects BC production (Jung et al., 2005; Zywicka et al., 2015).

The great pH to BC production depends on the micro producer organisms and it appears between four and seven pH, although the greatest BC production was at 6.5 pH (Son; Heo, 2001). To avoid contamination during the cultivation, some enterprises that uses this polymer material for biomedical purposes, chooses to operate between 4 and 4.5 pH (Jonas and Farah, 1998). Son and Heo (2001) studied the temperature's effect on bacteria *Acetobacter sp*. A9 productivity at 20 ^{0}C and 40 ^{0}C. The best value found was at 30 ^{0}C. The temperature does not only affect the productivity, but also the morphology and the crystalline polymer structure. The dissolved oxygen inside of the culture is essential to cellular metabolism, yield and final polymer quality (Shirai et al., 1994).

To BC production, new nitrogen and carbon sources having a high performance and low cost must be found (Çakar et al., 2014). Agro-industries residues (food waste, wheat straw, fruits waste, glycerol residues and cotton-based textile waste) can be used for BC production, aiming to reduce the costs and as environmental friendly option (Li et al., 2015). Amorim et al. (2019) affirms that the use of such residual material it is not only environmentally friendly but also helps to reduce the pollution associated to industrial waste disposal.

Methods of producing cellulose from microorganisms are being presented as a sustainable alternative such as raw material for several artifacts and industrial applications. BC is an extracellular polymer formed by linear coupling of sugar glucopyranose monomers and synthesized by strains of *Gluconacetobacter xylinus* and other bacteria (Shoda and Sugano, 2005). Acetobacter strains can be grown in the laboratory, found in fruits, is non-pathogenic and can have high cellulose production yield (Moosavi-Nasab and Yousefi, 2011; Klemm et al.,2009). When fermented in a culture rich in polysaccharides, these bacteria produce BC films that form a network of randomly organized fibrils and nano fibrils (Okiyarna et al.,1992) The BC has the same chemical structure of vegetable cellulose; however, it stands out in water retention capacity, mechanical strength and purity (Iguchi et al., 2000).

Recent research uses agricultural residues in the production of BC, which aims to optimize the process and the properties of the biomaterial, in addition to reducing the production cost. The olive oil, corn bagasse, fermented grape juice, wheat straw, molasses, cotton waste and effluent from the production of sweets, exhibit variable sugar content and are used as a source of carbon and nitrogen in the optimization process (GOMES et al., 2013; LI et al, 2015). Replacing the HS standard culture means for agro-industrial waste reduces the cost of production by more than 78%, in addition to minimize the environmental impacts (Costa et al. 2017).

Life cycle analyses (LCA) is the tool used to evaluate the impact caused on the environment. To this evaluation it is necessary assemble an inventory of relevant input and output, analyze the possible environmental impact associated to the input and final product, and interpret the results on each inventory phase and impact regarding the studies goal. Regarding the recycle analysis, it helps to determine if the reduction, recycling and recovery or disposal of the residues represents a viable environment option. In addition, analysis the energy consumption, the raw materials used, and the solid, liquid and gaseous waste produced in each process step. It can be especially useful in comparing the environmental impact of a recycled product with new materials (Arvanitoyannis, 2008; Passuello et al., 2014).

SimaPro®, created by PRé Consultants, is one of the most used software in the world to perform Life Cycle Analysis. In addition to following the recommendations of ISO 14040, it is possible to explore an immense database, to model study scenarios (real and proposed), to calculate the environmental impacts and emissions generated by the scenarios under study and to compare them. In Brazil, the license is made available by the company ACV Brasil for a fee, in this study the Faculty license was made available for free, version 8.5.0.0 of 2017.

The Life Cycle Analysis (LCA) is presented in NBR ISO 14040, which is a tool that evaluates the environmental aspects resulting from anthropic interventions. According to the ISO 14040 series, the LCA is structured in four phases: goal and scope definition, inventory analysis, impacts assessment and interpretation. Irrespective of LCA type, the analyses process is the same. It includes environmental LCA (E-LCA), social LCA (S-LCA) and life cycle sustainability assessment (LCS A). It identifies the environmental impacts caused during the design and production of a product, from the extraction of natural resources, manufacture until the waste's disposal. For a better evaluation, it is possible to delimit the study to evaluate which impacts will be studied and focus on a specific area for analysis. In other words, this delimitation works as a system boundary, in which only the environmental impact within this limit are taken under consideration, anything else is disregard. This are called "cut off" and its purpose is to make the analysis more feasible, making it possible to be specific. The LCA tool enables the identification of sustainable solutions to minimize impacts to the environment during all stages of production and the product lifetime (Brasil, 2009; Brasil 2009, Ferreira, 2004).

To analyze the environmental Life Cycle for BC production in standard HS (Hestrin and Schramm, 1954) and alternative means (Costa et al. 2017) was the objective of the study.

2. Methods

Step 1 - Microorganism and culture conditions - for BC production, a strain of *Gluconacetobacter hansenii* UCP1619, obtained from the culture collection of Nucleus of Resource in Environmental Sciences, Catholic University of Pernambuco, Brazil, was used. The standard culture medium used in the experiments was the HS described by Hestrin and Schramm (1954) and modified by Hungund and Gupta (2010). The liquid medium contained 2.0 % glucose (w/v), 0.5 % yeast extract (w/v), 0.5 % peptone, 0.27 % Na_2HPO_4 (w/v), and 0.15 % citric acid (v/v). For the production of BC, the bacterium was cultivated in an alternative medium (Costa et al, 2017), in which the carbon source was reduced by 25% and synthetic nitrogen was completely replaced with corn steep liquor (CSL): 1.5 % glucose (w/v), 2.5 % CSL (w/v), 0.27 % Na_2HPO_4 (w/v), and 0.15 % citric acid (v/v).

Step 2 – 3 % of the inoculum produced with *Gluconacetobacter hansenii* was inoculated in a semi-capped glass vessel (250 mL) containing 100 mL of the different liquid media, and then statically incubated at 30 °C (BC produced in HS medium) and at room temperature (BC produced in alternative medium formulated CSL) for 10 days (Gomes et al., 2013; Wu et al., 2014). After cultivation, the BC pellicles were sent for cleaning, purification (NaOH 1.0 M) as well as the determination of thickness, hydrated mass, and dry mass.

Step 3 - Then, the pellicles were washed with deionized water several times to warrant the complete remove the alkali, leaving the pellicle at neutral pH. The BC produced in HS medium was dried in an electric oven while the one produced in alternative medium dried in room temperature, until reaching a constant mass.

Step 4 - LCA, the structuring of the BC production study was analyzed as an alternative of sustainable raw material to replace vegetable cellulose (VC) in the artifact projects.
The development of the LCA methodology followed the recommendations of NBR ISO 14040 (BRAZIL, 2009) and NBR ISO 14044 (Brasil, 2009). Beginning with the definition of the two scenarios for the study, after an identification of all inputs used for BC production. Scenario 1 was defined as that where BC pellicle production takes place in HS medium and scenario 2 was that where BC pellicle production takes place in an alternative medium containing CSL.
All the necessary inputs for both fermentative processes were listed. (Table 1).

Table 1: Actions and inputs required for both BC pellicle production scenarios

Culture media used in BC production		
Input	**HS medium** (HESTRIN and SCHRAMM, 1954)	**Alternative medium** (COSTA et al., 2017)
Distilled water (mL)	1,000	1,000
Glucose (g)	20.0	15.0
Peptone (g)	5	-
Yeast Extract (g)	5	-
Disodium phosphate (Na2HPO4) (g)	2.7	2.7
Citric acid monohydrate (CAH) (g)	1.5	1.5
CSL (g)	-	25.00
Autoclave sterilization (1,500 W)	15 min at 121 °C	15 min at 121 °C
Inoculum preparation in laminar flow hood (1,000 W)	10 min	10 min
Fermentation in the greenhouse (1,100 watts, in static condition.	10 days at 30 °C	-
Rinse in running water and neutralize with 1.0 M NaOH in a hot plate (15,000 W) then wash in running water to pH 7.	30 min at 80 °C	30 min at 80 °C
Electric oven drying (15,000 W)	12 hour at 60 °C	-

Source: Authors (2019).

Step 5 – The functional unit and the reference flow for the production of 1 kg BC pellicles was chosen for both scenarios.

Step 6 - Life Cycle Inventory Analysis (LCI). First, the data collection was structured, and then were made calculations and measurements of the inputs and outputs regarding important information throughout the production process of BC pellicles considering the two different culture media.

Step 7 – Simulation in SIMAPRO® college version software. The significance of potential environmental impacts was assessed from the Life Cycle Inventory that analyzed:

- The results were interpreted taking under consideration specific environmental indicators using the fReCiPe Midpoint (I) analyses method considering that, this method ranks the obtained results.
- Environmental impact categories are described in (Table 1).

Table 2: Environmental impact categories for the two production scenarios of BC pellicles in culture medium, HS and alternative medium containing CSL

Environmental Impact Category	Environmental Damage Assessment	Unit of measurement
Global warming	atmospheric emissions	(kg CO_2 eq)
Freshwater Eutrophication	eutrophication in aqueous medium	(kg P eq)
Freshwater ecotoxicity	toxicity of chemicals in the aqueous environment	(kg 1,4-DCB)
Human carcinogenic toxicity	carcinogenic influence	(kg 1,4-DCB)

Source: Authors (2019).

3. Results and discussion

During the compound's identification present in HS culture media and alternative medium containing CLS was observed that the industrial residue was able to replace the synthetic carbon nitrogen sources that are added to the culture media. It made possible to reduce the production cost in more than 60 % as mentioned by (Costa et al. 2017). The need for electric energy for fermentation and drying of pellicles, produced in HS, increases the cost of biomaterial production and the environmental impact. The experiments were carried out under sterile conditions (Figure 1).

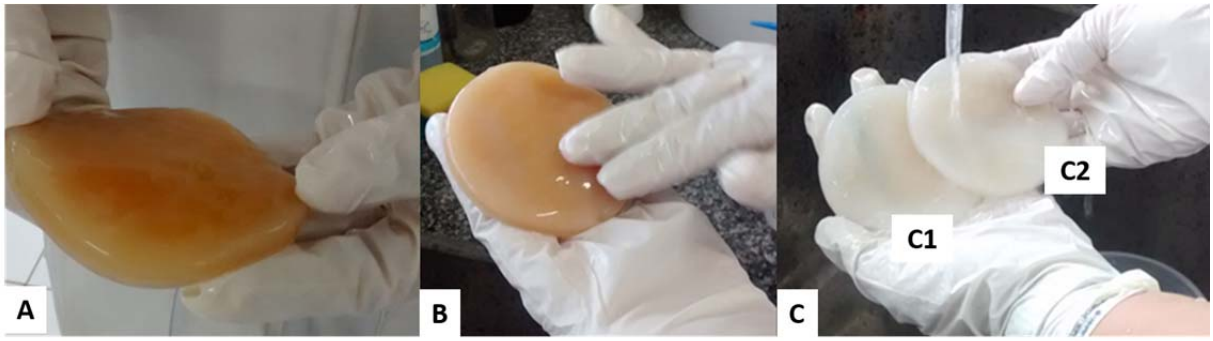

Figure 1 Macrograph of BC produced after 10 days of static cultivation in HS medium (A) and in alternative medium contend CSL (B). Pellicles after NaOH treatment in, HS medium (C1) and in alternative medium formulated CSL (C2).

The BC produced by the HS method and the alternative mean present similar characteristics when observed to the naked eye, such as, color, visual and tactile texture and Scanning Electron Microscopy (SEM) to the distribution and organization of micro and nanofibrils. In the mechanical assay, the obtained results had very close value for the elastic behavior, deformation and maximum tensile stress for the hydrated and dry BC film. In addition to the index of crystallinity and thermal degradation. By showing that the BC produced with the residue presented a cost reduction of more than 80 %, it was identified that the biomaterial has properties for sustainable industrial application (Amorim et al, 2019; Galdino et al, 2019; Costa et al, 2019).

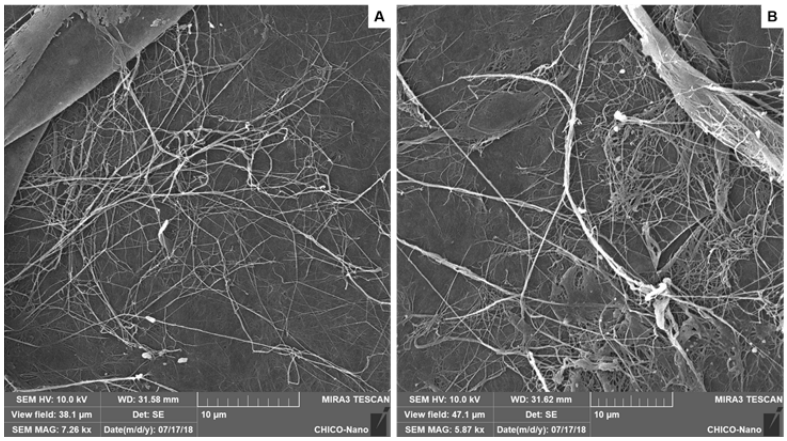

Figure 2 SEM images of BC pellicles obtained after 10 days of fermentation in HS medium (A) and alternative medium formulated with CSL (B).

In SIMAPRO®, it was observed that the percentage reductions in the categories of global warming (kg CO_2 eq), freshwater eutrophication (kg P eq), freshwater ecotoxicity (kg 1,4-DCB) and human carcinogenic toxicity (kg 1, 4-DCB) on the environment without the CSL and the one the contained it were respectively: -99.99 %, 98.76 %, 99.99 % and 98.81 %. This happened because the BC membranes were cultivated in alternative medium containing CS. This confirms that, the electric greenhouse used ate 35 ºC during the ten days of fermentation, increases the environmental impact due its high electric energy use, because the power consumption raises the percentage of the analyzed categories.

4. Conclusions

The culture medium that uses CSL to produce BC according to the LCA shows a considerable reduction of environmental impacts. Considering a production for industrial application of the biomaterial the reduction of production costs can also be high.

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