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iCons IDEA SLAM 2023 – The Problem with Unsustainable Chocolate Production

Dried and fermented cacao beans – used to make chocolate – are the only commercial product derived from the cacao fruit. Cacao beans correspond to only 8-10 wt % of the fruit; the remaining biomass is typically disposed of in farm fields, causing environmental problems such as spread of disease and water pollution. This is a huge problem considering that **60 million tons** of cacao fruit are grown each year across the globe, with Africa producing about 2/3 of global cacao, and South America about 20%. What's more, the wasted biomass has many promising applications.

We want to start a cacao revolution, using science and innovation to discover how to utilize the entire cacao fruit. We envision that wholesome strategies for biomass utilization from commercial crops will soon become the norm – with new generations of consumers driven by the desire for sustainability, fair-trade, direct trade, and reduced environmental impacts.

We are Nextcoa – a startup company based in Colombia, South America focused on developing a technical "toolbox" for processing cacao fruits and producing 100% cacao-based products. There are many possible applications of the residual biomass, but we need your help to brainstorm on both **end-user applications** and ways to **transform the biomass** to increase its value. Please check out all the links and be ready to tackle these problems with us. Here's some more reading to bring you up to speed on this revolutionary field:

- "Overview of Bacterial Cellulose Production and Application," (2014)
- "Cellulose Biosynthesis Using Simple Sugars in Residual Cacao Mucilage Exudate," (2021)

Marianny Y. Combariza, PhD. Director of the Center for Environmental Studies (CEIAM) and Professor, School of Chemistry at Universidad Industrial de Santander (UIS) Bucaramanga. Colombia. Prof Combariza's research interests include Analytical Chemistry (Mass Spectrometry), Materials Chemistry, and Nanotechnology. Current research focuses mainly on HRMS analysis of complex mixtures (crude oil, asphaltenes, natural pigments); development of electron-transfer MALDI matrices for analysis of labile and thermally unstable molecules (metal complexes, natural and synthetic pigments, among others); biosynthesis, isolation, characterization and modification of nano cellulose from residual lignocellulosic biomass; development of cellulose-based functional materials for industrial applications. Regarding the latter field Prof Combariza is actively involved in designing cellulose-based functional composites, with transition metal nanoparticles, for environmental remediation of contaminated waters, mainly with recalcitrant organic compounds such as textile dyes. In addition, she uses analytical techniques to establishment degradative pathways of these organic pollutants.

Cristian Blanco-Tirado, PhD. Professor of Chemistry at Universidad Industrial de Santander (UIS) Bucaramanga. Colombia. Prof. Blanco-Tirado research interests include materials science, wasted biomass, industrial processes thermodynamics, Materials Chemistry, and Nanotechnology. Current research focuses mainly on lignocellulosic materials; development of cellulose-based functional materials for industrial applications. Materials and food chemistry. Entrepreneurship, pragmatic research and development.

How West Africa Can Reap More Profit From the Global Chocolate Market

Resource-rich countries like Ghana are often cut out of lucrative parts of the business like manufacturing. The “fairchain movement” wants to change that.

By Patricia Cohen
Reporting from Accra and Amanase, Ghana

Sept. 28, 2023

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The first leg of the 35-mile journey from Ghana’s capital city, Accra, to the Fairafric chocolate factory in Amanase on the N6 highway is a quick ride. But after about 30 minutes, the smoothly paved road devolves into a dirt expanse without lanes. Lumbering trucks, packed commuter minivans, cars and motorcycles crawl along craggy, rutted stretches bordered by concrete dividers, muddy patches and heaps of rock.

The stopgap roadway infrastructure is one of the challenges Fairafric has had to navigate to build a factory in this West African country. The area had no fiber-optic connection to Ghana’s telecommunications network. No local banks were interested in lending the company money. And it required the personal intervention of Ghana’s president before construction could even begin in 2020.

The global chocolate industry is a multibillion-dollar confection, and Africa grows 70 percent of the world’s raw cocoa beans. But it produces only 1 percent of the chocolate — missing out on a part of the business that generates the biggest returns and is dominated by American and European multinationals.

The Fairafric chocolate factory powered by solar energy in Amanase, Ghana. The company aims to create stable, well-paying jobs. Francis Kokoroko for The New York Times

Capturing a bigger share of the profits generated by chocolate sales and keeping them in Ghana — the second-largest cocoa exporter behind Ivory Coast — is the animating vision behind Fairafric. The aim is to manufacture the chocolate and create stable, well-paying jobs in the place where farmers grow the cocoa.

Many developing countries are lucky to have large reserves of natural resources. In Ghana, it’s cocoa. In Botswana, it’s diamonds. In Nigeria and Azerbaijan, it’s oil. But the commodity blessing can become a curse when the sector sucks up an outsize share of labor and capital, which in turn hampers the economy from diversifying and stunts long-term growth.

“Look at the structure of the economy,” Aurelien Kruse, the lead country economist in the Accra office of the World Bank, said of Ghana. “It’s not an economy that has diversified fully.”

The dependency on commodities can lead to boom-and-bust cycles because their prices swing with changes in supply and demand. And without other sectors to rely on during a downturn — like manufacturing or tech services — these economies can crash.

“Prices are very volatile,” said Joseph E. Stiglitz, a former chief economist at the World Bank. In developing nations dependent on commodities, economic instability is built into the system.

Workers making the chocolate products. By keeping manufacturing in Ghana, Fairafric supports other local businesses. Francis Kokoroko for The New York Times

A batch of chocolate bars being inspected . . . Francis Kokoroko for The New York Times

. . . and packaged at the Fairafric chocolate factory. Francis Kokoroko for The New York Times

But creating industrial capacity is exceedingly difficult in a place like Ghana. Outside large cities, reliable electricity, water and sanitation systems may need to be set up. The suppliers, skilled workers, and necessary technology and equipment may not be readily available. And start-ups may not initially produce enough volume for export to pay for expensive shipping costs.

Fairafric might not have succeeded if its founder and chief executive — a German social-minded entrepreneur named Hendrik Reimers — had not upended the status quo.

The pattern of exporting cheap raw materials to richer countries that use them to manufacture valuable finished goods is a hangover from colonial days. Growing and harvesting cocoa is the lowest-paid link in the chocolate value chain. The result is that farmers receive a mere 5 or 6 percent of what a chocolate bar sells for in Paris, Chicago or Tokyo.

Mr. Reimers's goal is aligned with the "fairchain movement," which argues that the entire production process should be in the country that produces the raw materials.

The idea is to create a profitable company and distribute the gains more equitably — among farmers, factory workers and small investors in Ghana. By keeping manufacturing at home, Fairafric supports other local businesses, like the paper company that supplies the chocolate wrappers. It also helps to build infrastructure. Now that Fairafric has installed the fiber optic connections in this rural area, other start-up businesses can plug in.

Cocoa pods harvested in a cocoa farm in Ghana. Francis Kokoroko/Reuters

A worker from Fairafric chocolate factory visiting a cocoa farm in the Budu community. Francis Kokoroko for The New York Times

The last few years have severely tested the strategy. Ghana's economy was punched by the coronavirus pandemic. Russia's invasion of Ukraine fueled a rapid increase in food, energy and fertilizer prices. Rising inflation prompted the Federal Reserve and other central banks to raise interest rates.

In Ghana, the global headwinds exacerbated problems that stemmed from years of excessive government spending and borrowing.

As inflation climbed, reaching a peak of 54 percent, Ghana's central bank raised interest rates. They are now at 30 percent. Meanwhile, the value of the currency, the cedi, tumbled against the dollar, more than halving the purchasing power of consumers and businesses.

At the end of last year, Ghana defaulted on its foreign loans and turned to the International Monetary Fund for emergency relief.

“The economic situation of the country has not made it easy,” said Frederick Affum, Fairafric's accounting manager. “Every kind of funding that we have had has been outside the country.”

Even before the national default, Ghana's local banks were drawn to the high interest rates the government was offering to attract investors wary of its outsize debt. As a result, the banks were reluctant to invest in local businesses. They “didn't take the risk of investing in the real economy,” said Mavis Owusu-Gyamfi, the executive vice president of the African Center for Economic Transformation in Accra.

“The economic situation of the country has not made it easy,” said Frederick Affum, accounting manager at Fairafric. Francis Kokoroko for The New York Times

Fairafric started with a crowdsourced fund-raising campaign in 2015. A family-owned chocolate company in Germany bought a stake in 2019 and turned Fairafric into a subsidiary.

In 2020, a low-interest loan of 2 million euros from a German development bank that supports investments in Africa by European companies was crucial to getting the venture off the ground.

Then the pandemic hit, and President Nana Akufo-Addo closed Ghana's borders and suspended international commercial flights. The shutdown meant that a team of German and Swiss engineers who had been overseeing construction of a solar-powered Fairafric factory in Amanase could not enter the country.

So Michael Marmon-Halm, Fairafric's managing director, wrote a letter to the president appealing for help.

“He opened the airport,” Mr. Marmon-Halm said. “This company received the most critical assistance at the most critical moment.”

Both Ghana and Ivory Coast, which account for 60 percent of the world cocoa market, have moved to raise the minimum price of cocoa and expand processing inside their borders.

In Ghana, the government created a free zone that gives factories a tax break if they export most of their product. And this month, Mr. Akufo-Addo announced an increase in the minimum price that buyers must pay farmers next season.

Cocoa pods at a cocoa farm in the Budu community . . . Francis Kokoroko for The New York Times

. . . which reveal a pulpy white bean when cracked open. Francis Kokoroko for The New York Times

Fairafric, which buys beans from roughly 70 small farmers in the eastern region of Ghana, goes further, paying a premium for its organically grown beans — an additional $600 per ton above the global market price.

Farmers harvest the ripe yellow pods by hand, and then crack them open with a cutlass, or thick stick. The pulpy white beans are stacked under plantain leaves to ferment for a week before they are dried in the sun.

On the edge of a cocoa farm in Budu, a few minutes from the factory, a bare-bones, open-sided concrete shed with wooden benches and rectangular blackboards houses the school. Attendance is down, the principal said, because the school has not been included in the government's free school feeding program.

The factory employs 95 people. They have health insurance and are paid above the minimum wage. Salaries are pegged to the dollar to protect against currency fluctuations. Because of spotty transportation networks, the company set up a free commuter van for workers. Fairafric also installed a free canteen so all the factory shifts can eat breakfast, lunch or dinner on site.

Mr. Marmon-Halm said the company was looking to raise an additional $1 million to expand. He noted that the chocolate industry generated an enormous amount of wealth.

But "if you want to get the full benefit," he said, "you have to go beyond just selling beans."

Students by a stream in the Budu community, a cocoa farming village. Francis Kokoroko for The New York Times

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Overview of Bacterial Cellulose Production and Application

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Abstract

Bacterial cellulose (BC), produced by aerobic bacteria received ample of attention due to its unique physiochemical properties compared to plant cellulose. Intense researches on BC mainly focus on biosynthetic process to achieve low-cost preparation and high cellulose production. BC has been used as biomaterial for medical field, electrical instrument and food ingredient. However, BC alone has limited capabilities to fulfil current demand on high-performance biomaterials. Hence, BC composite has been introduced to enhance BC properties through addition of reinforcement materials. This review discusses current knowledge in fermentation process and potential application of BC including its application in food industry.

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Keywords: Bacterial cellulose; a*cetobacter xylinum*; fermentation; composite material

1. Introduction

Cellulose is the most abundant, inexpensive and readily available carbohydrate polymer in the world, traditionally extracted from plants or their wastes. This polymer normally branches with hemicellulose and lignin has to undergo unhealthy chemical process with harsh alkali and acid treatment to obtain the pure product (Sun, 2008). Increasing demand on derivatives of plant cellulose had increased wood consumption as raw material, causing deforestation and global environmental issue (Park et al., 2003).

Although plant is the major contributor of cellulose, various bacteria are able to produce cellulose as an alternative source. Bacterial cellulose (BC) was initially reported by Brown (1988) who identified the growth of unbranched pellicle with chemically equivalent structure as plant cellulose. Due to BC structure that consist only

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glucose monomer, it exhibits numerous great properties such as unique nanostructure (Chen et al., 2010), high water holding capacity (Saibuatong and Phisalaphong, 2010), high degree of polymerization (Dahman et al., 2010), high mechanical strength (Castro et al., 2011) and high crystallinity (Keshk, 2014). The discovery from previous researches had clearly shown that BC and its derivatives have tremendous potential and provide a promising future in various fieldssuch as biomedical, electronic and food industrial (Shah et al., 2013; Zhu et al., 2010).

BC is produced by acetic acid bacteria in both synthetic and non-synthetic medium through oxidative fermentation. *Acetobacter xylinum* is the most studied andthe most efficient BC producer (El-Saied et al., 2004) that manage to assimilate various sugars and yields high level of cellulose in liquid medium (Ross et al., 1991; Sani and Dalman 2010; Moosavi-Nasab and Yousefi, 2011). This aerobic gram-negative bacteria actively fermented at pH 3-7 and temperature between 25 and 30˚C using saccharides as carbon source (Castro et al., 2011). Rivas et al. (2004) reported that almost 30% of bacterial fermentation is belong to the cost of fermentation medium. High cost and low-yield production has limited the industrial production of BC and its commercial application. Therefore, it is important to look for a new cost-effective carbon source with shorter fermentation process for high yield BC production.

2. Bacterial cellulose structure

BC exists as a basic structure of fibril that consists of β-1→4 glucan chain with molecular formula $(C_6H_{10}O_5)n$. The glucan chains are held together by inter- and intra- hydrogen bonding (Ul-Islam et al., 2012) (Fig. 1). Microfibrils of BC were first described by Muhlethalerin 1949 and about 100 times smaller than plant cellulose (Chawla et al., 2009; Gayathry and Gopalaswamy, 2014).

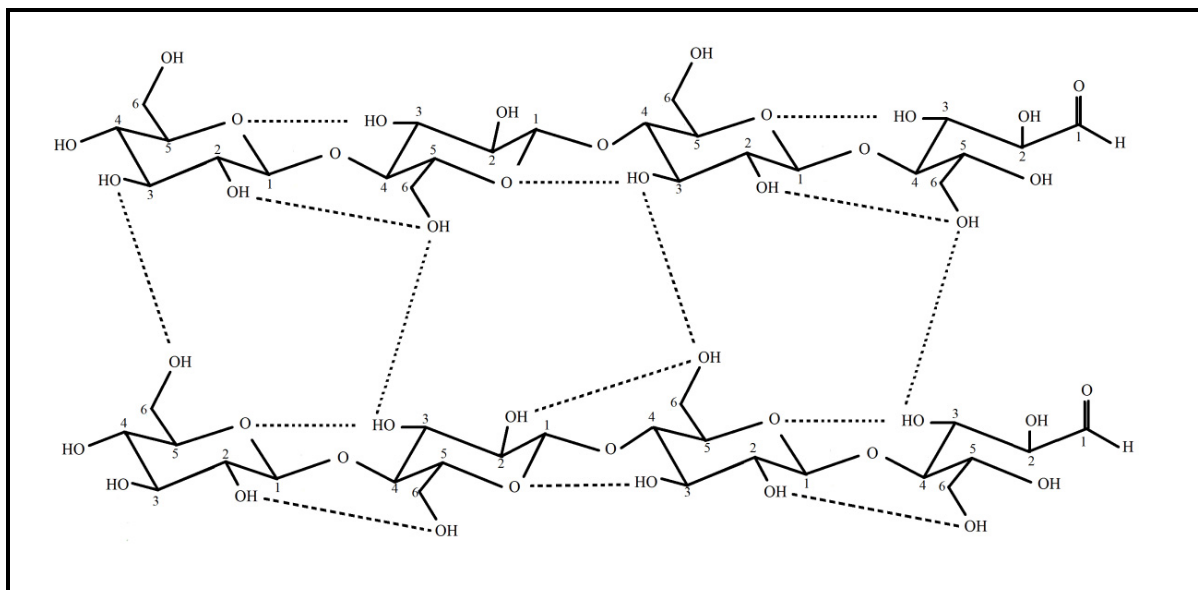

Fig. 1. Inter- and intra-hydrogen bonding of bacterial cellulose (edited from Festucci-Buselli et al. 2007).

The fibrous network of BC is made of three dimensional nanofibres that well-arranged, resulting in formation of hydrogel sheet with high surface area and porosity. *Acetobacter xylinum* produces cellulose I (ribbon-like polymer) and cellulose II (thermodynamically stable polymer) as described in Fig. 2 (Chawla et al., 2009). During the synthesis process, protofibrils of glucose chain are secreted through bacteria cell wall and aggregate together forming nanofibrils cellulose ribbons (Ross et al., 1991). These ribbons construct the web shaped network structure of BC with highly porous matrix (Dahman, 2009; Maria et al., 2010). The cellulose formed has abundant surface of hydroxyl groups that explaining it as hydrophilicity, biodegradability, and chemical-modifying capacity (Klemm et al., 2005). Further mechanism of BC synthesis was clearly explained by Chawla et al. (2009).

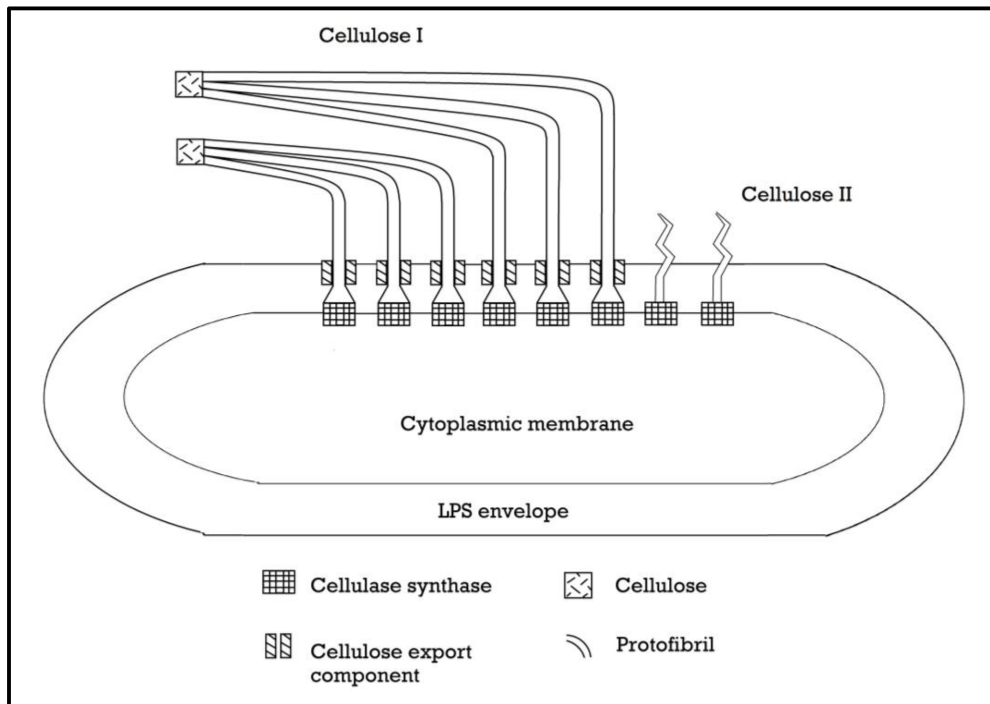

Fig. 2. Production of cellulose microfibrils by *Acetobacter xylinum* (Chawla et al. 2009).

3. Fermentation process

3.1 Culture condition

Fermentation of BC production is conducted in either static, agitated or stirred conditions. Different forms of cellulose are produced under these conditions. Three dimensional interconnected reticular pellicle was reported under static condition while both agitated and stirred condition produced irregular shape sphere-like cellulose particle (SCP) (Tanskul et al., 2013). The process of cellulose formation under static condition is regulated by air supply from medium surface and the yield depends on the carbon source concentration moderately (Budhiono et al., 1999). The increasing of growth time will increase the formation of BC along with hydrogen and C-H bonding (Sheykhnazari et al., 2011). Synthesis of BC reach its limit when the pellicle growth downward and entraps all bacteria. Bacteria become inactive due to insufficient oxygen supply (Borzani and Desouza, 1995).Semi-continuous process in static condition is recommended at industrial scale as it manage to increase BC productivity compared to continuous process (Çakar et al., 2014).

In contrast, due to low yield of the static production, most of cellulose used in commercial purpose is generated through agitated fermentation. Agitated condition causes formation of SCP, an irregular forms of cellulose either in fibrous suspension, spheres, pellets or irregular masses (Yan et al., 2008, Wu and Lia, 2008). The SCP has lower crystallinity, mechanical strength and degree of polymerization compared to pellicle from static culture (Shi et al., 2013). The altered microfibrils organization was proposed related to the disruption effects of aeration on the hydrogen bonds formations between cellulose (Bootten et al., 2008). Study by Hu et al. (2013) found that the number of SCP decreased with the increasing volume of inoculums while different initial glucose concentration only gave an impact to the mean diameters of SCP. However, the mechanism of SCP formation is still remain unknown.

3.2 Culture medium

In biorefinery concept, a progressive transition to economically renewable materials as feedstock for chemical, materials and fuels is predicted to occur due to depletion of fossil resources (Octave and Thomas, 2009). In order to find a new economical culture medium for industrial scale production of BC, many studies have focused on agriculture waste and industrial by-product as potential medium (Kurosumi et al., 2009; Gomes et al., 2013; Çakar et al., 2014). Some of them have been proven as beneficial carbon source for BC production such as waste beer yeast (Lin et al., 2014), dry oil mill residue (Gomes et al., 2013), thin stillage (Wu and Liu, 2012), grape skin (Carreira et al., 2011) and maple syrup (Zeng et al., 2011). Besides that, the use of such products gives a positive impact on corresponding industry by decreasing the environmental problems associate with disposal of waste. In addition, medium supplementation with nitrogen and phosphorus sources were confirmed would increase BC production (Gomes et al., 2013).

The usage of glucose as carbon source during BC production is associated with the formation of gluconic acid as by-product in the culture medium. This by-product will decreasethe pH of the culture and negatively affect the quantity of BC production. However the presence of antioxidant and polyphenolic compounds manages to inhibit the gluconic acid formation (Keshk and Sameshima, 2006).

4. Bacterial cellulose composite

BC has been applied in multiple field such as wound dressing (Muangman et al., 2011), blood vessel regeneration (Wippermann et al., 2009), and paper restoration (Santos et al., 2014). Although BC has unique properties, there is limitation that restricts its applications such as lack of antibacterial properties, optical transparency, and stress bearing capability. To overcome these limitations, BC composite has been introduced which consist of a matrix and reinforcement materials. BC owns a porous nature arrangement of fibres. It acts as matrix for housing a variety of particles from different reinforcement materials. The anchored reinforcement materials provide an additional properties to BC that impart its nature biological and physiochemical properties (Shah et al., 2013).

BC possess a potential as both matrix and reinforcement materials. Various BC composites have been synthesized through either in situ or ex situ methods. For the in situ method, reinforcement materials is added to the polymer during its synthesis (Saibuatong and Philsalaphong, 2010) while in the ex situ process, BC is impregnated with reinforcement materials (Ul-Islam et al., 2012). As shown in Table 1, various BC composites are synthesized with different function. BC composite can be either organic or inorganic material such as polymers (Kim et al., 2011), metal or metal oxides (Maneerung et al., 2007), solid materials (Meng et al., 2009) and nanomaterials (Yan et al., 2008).

Table 1. Bacterial cellulose composites and application.

No.	Application field	Reinforcement material	Function	References
1.	Electronic	Graphite nanoplatelet	Electrical conductivity	Zhou et al., 2013
2.	Electronic	Poly-4-styrene sulfonic acid	Redox flow battery	Gadim et al., 2014
3.	Biomedical / Industrial	Chitosan	Nanofilm	Fernandes et al., 2009
4.	Biomedical	Hydroxyapatite	Bone tissue engineering	Tazi et al., 2012
5.	Biomedical	Silver nanoparticles	Antimictobial wound dressing	Wu et al., 2014, Zhang et al., 2013
6.	Biomedical	Paraffin	Bone scaffolding	Zaborowska et al., 2010
7.	Electronic	Polyurethane	Film substrate of light emitting diod	Ummartyotin et al., 2012.

5. Application of bacterial cellulose in food industry

BC is traditionally used to make nata de coco, an indigenous dietary fiber of South-East asia that served as gelatinous cube. Nata de coco has textural properties like chewy, soft and smooth surface. It has no cholesterol, low in fat and low calories. During the production process, BC was synthesized in static culture of coconut water (Jagannath et al., 2008). Coconut water served as carbon source for *Acetobacter xylinum* and later converted to extracellular cellulose (Cannon and anderson, 1991). The thick sheet of cellulose was washed, boiled and cooked in sugar syrup for food applications such as desserts, fruit cocktails and jellies. High fiber supplement mixture of nata

de coco and cereal was reported able to reduced lipid level of consumer (Mesomya et al., 2006). On the other hand, nata de coco is also a very promising medium for continuous bioethanol production in term of structural strength and cost effectiveness (Montealegre et al., 2012).

Packaging plays an important role to protect and preserve the food. Bio-based materials in packaging industry is more preferable nowadays due to concern arise on non-biodegradable packaging impact to the environment. BC has been identified as one of the suitable material (Tang et al., 2012) as it consist of fine network, biodegradable (Sonia and Dasan, 2013), and high water resistance performance (Arrieta et al., 2014). Even BC is an excellent choice for food packaging, unfortunately it has no antibacterial and antioxidant properties to prevent food contamination. Therefore,BC composites are used to gain these properties (Gao et al., 2014). Further BC application are summarized in Table 2.

Table 2. Application of modified bacterial cellulose (BC) and its composites in food industry.

No.	Materials	Function	Types of food	References
1.	BC/nisin	Antimicrobial food packaging	meat	Nguyen et al., 2008
2.	BC/polylysine	Biodegradable food packaging	sausage	Zhu et al., 2010
3.	BC	Emulsifier	surimi	Lin et al., 2011
4.	Carboxymethycellulose	Regulate gough rheology	Flour dough	Correa et al., 2010
5.	Hyroxypropyl methyl cellulose	Texture enhancer	Whipped cream	Zhao et al.,2009
6.	Methyl cellulose	Enhancing shelf life	Egg	Suppakul et al., 2010
7.	Methyl cellulose	Enhance bioavailability	Vitamin C	Perez et al., 2013

6. Conclusions

Acetic acid bacteria are capable to replace plant as an alternative source of cellulose through oxidative fermentation under static, agitated and stirred conditions. However, high cost and low-yield production have limits its commercial applications and industry potentials. Therefore, researches have focus on agriculture waste and industrial by-product as new cost-effective carbon sources. Besides that, the modification and incorporation of particles in BC matrix enhance its nature physiochemical properties and bring up the new opportunity for applications.

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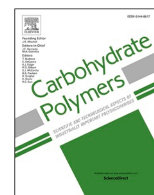

Cellulose biosynthesis using simple sugars available in residual cacao mucilage exudate

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ABSTRACT

Worldwide only 8% of the biomass from harvested cacao fruits is used, as cacao beans, in chocolate-based products. Cacao mucilage exudate (CME), a nutrient-rich fluid, is usually lost during cacao beans fermentation. CME's composition and availability suggest a potential carbon source for cellulose production. CME and the Hestrin and Schramm medium were used, and compared, as growth media for bacterial cellulose (BC) production with *Gluconacetobacter xylinus*. CME can be used to produce BC. However, the high sugar content, low pH, and limited nitrogen sources in CME hinder *G. xylinus* growth affecting cellulose yields. BC production increased from 0.55 ± 0.16 g L^{-1} up to 13.13 ± 1.09 g L^{-1} after CME dilution and addition of a nitrogen source. BC production was scaled up from 30 mL to 15 L, using lab-scale experiments conditions, with no significant changes in yields and production rates, suggesting a robust process with industrial possibilities.

1. Introduction

Agriculture is on the rise to feed a hungry world; however, food production circularity is still low. Annually, approximately one-third of the food produced (1.3 billion tons/year) is discarded during processing, distribution, and consumption (Güzela & Akpınarb, 2020; Santos et al., 2020). Another agricultural growth outcome, residual -or lignocellulosic- biomass, is quickly becoming an abundant resource for materials and energy production. Thus, modern agricultural circular economy schemes seek synergism between food production (primary use) and fuel or materials production (secondary and tertiary uses), with the ultimate goal of increasing economic profitability while reducing environmental impacts. From the materials production point of view, residual agricultural biomass, particularly from fruit crops, could be a viable carbon source for biopolymers production due to its composition, wide availability, and low cost (Kumar et al., 2019; Kurosumi et al., 2009) Many researchers agree that in the near future, this type of biomass will become the best alternative for the production of bacterial cellulose (BC) in terms of yields, cost, and energy consumption (Carreira et al., 2011; Kumar et al., 2019; Perna Manrique et al., 2018).

BC is structurally identic to plant-derived cellulose. The polymer is composed of repeating cellobiose units linked by β-1,4 glycosidic bonds. The 3D structure of BC consists of an ultrafine network of high purity cellulose nanofibers (3–8 nm) with an exceptional water retention capacity (Campano et al., 2016). BC biofilms are non-toxic, hypoallergenic, biocompatible, and biodegradable. Also, they exhibit high mechanical resistance, elasticity, and degradation temperatures higher than plant cellulose (Campano et al., 2016). BC biosynthesis typically involves using a standard culture medium similar to the one developed by Hestrin and Schramm (HS) in 1954. The process involves static or dynamic fermentation with bacterial strains of various genera (e.g., *Enterobacter*, *Escherichia*, *Lactobacillus*, *Salmonella*, *Rhizobium*, *Agrobacterium*, *Sarcina*, *Achromobacter*, *Alcaligenes*, *Azotobacter*, and *Gluconacetobacter*) (Khan et al., 2019; Vazques et al., 2013), with the ability to produce the biopolymer. However, the *Gluconacetobacter* strains are preferred for industrial (large-scale) BC production from a wide range of carbon sources such as glucose, saccharose, glycerol, fructose, or mannitol (Mikkelsen et al., 2009; Yang et al., 2019).

Owing to its physicochemical properties and high purity, BC offers a wide variety of applications in the cosmetic, textile, food, paper, pharmaceutical, and biomedical industries (Esa et al., 2014). BC is used directly in wound dressings, burn treatments, medical devices, tissue regeneration, biosensing materials, and scaffolding for drug delivery. However, despite its potential for many commercial applications, the

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low rates of large-scale production and the high costs of synthetic culture media, such as the HS medium, limit the uses of BC (Basu et al., 2018; Campano et al., 2016).

The culture media used in the production of BC is typically a water-based liquid containing sources of carbon (simple sugars), nitrogen, micronutrients, and vitamins necessary to support bacterial growth (Chawla et al., 2009; Lin et al., 2013). For *G. xylinus* cultures, the literature describes a wide variety of pure carbon (e.g., glucose, saccharose, fructose, and glycerol), nitrogen (e.g., yeast extract, casein hydrolysate, ammonium sulfate, soy extract, peptone, and sodium glutamate) sources (Jagannath et al., 2008; Jung et al., 2010; Keshk & Sameshima, 2005; Mikkelsen et al., 2009; Pourramezan et al., 2009; Ramana et al., 2000) and additives such as sodium citrate, ethanol, vegetable oil, and ascorbic acid, among others (Keshk, 2014; Li et al., 2012; Żywicka et al., 2018). Unconventional carbon sources are also tested to increase BC production and decrease costs. Alternative sugar-rich carbon sources for the production of BC include molasses (Jaramillo et al., 2015), maple syrup (Zeng et al., 2011), coconut water (Hungund, 2013), fruit juices (Kumar et al., 2019; Kurosumi et al., 2009; Perna Manrique et al., 2018), corn liquor (El-Saied et al., 2008), beet, cheese, whey, and potato processing effluents (Thompson & Hamilton, 2001), as well as residual agricultural biomass (Carreira et al., 2011).

In Colombia, cacao is a traditional crop, now the focus of a lot of attention because it is used as a substitute crop to illegal cocaine plantations to provide economically-sound alternatives to farmers in the country. As in any other cacao-producing countries, in Colombia, cacao beans are transformed under a linear economy model where only roughly 8 to 10% of the biomass from the harvested cacao fruit ends up in the final product as chocolate. Cacao beans production involves cacao fruit harvest, followed by pod opening, fresh beans extraction, beans fermentation in heaps or wooden crates, sun drying, and storage of the fermented/dried beans (Vásquez et al., 2019). The first three steps are performed on the field, where the empty cacao pod husk and the placenta (a supporting tissue holding the beans inside the pod) are typically left to decompose. Thus, three primary by-products originate from the initial cacao fruit processing: cacao pod husk (CH), fresh cacao beans (CB), and cacao placenta (CP). Fresh CH is the most abundant material with an average of 65 ± 1.2 wt%, followed by fresh CB with 25 ± 1.4 wt%, and CP with 2.6 ± 1.4 wt% (Campos-Vega et al., 2018). However, the cacao fruit can offer other by-products such as cacao mucilage exudate or sweatings (CME). CME is a translucent liquid that seeps from the white pulp or mucilage surrounding the fresh cacao beans and is mainly composed of simple carbohydrates (saccharose, glucose, and fructose), pectins, protein, and trace elements such as calcium, potassium, sodium, and magnesium. CME is acidic, with a pH of 3.6 to 3.8, due to high concentrations of carboxylic acids (Puerari et al., 2012). Approximately 30 to 50 L of CME, depending on the cacao variety, can be produced per ton of fresh cacao beans (3–5 wt%); however, CME is generally lost as a lixiviate during cacao beans fermentation. CME composition and availability makes it a potential carbon source for the biosynthesis of cellulose.

In this contribution, CME was tested as a carbon source for BC production in a static fermentation process using the bacterial strain *Gluconacetobacter xylinus* (ATCC®23768™). The standard media Hestrin and Schramm (HS) was used as a control to estimate the performance of CME in BC production. Comprehensive physicochemical characterization of CME allowed growth media formulation and monitoring. Also, we followed the BC biosynthesis process in terms of pH evolution, consumption of mono- and disaccharides, and BC production. We also report on the physicochemical, thermal, and morphological properties of BC films produced using residual CME and compare these characteristics with BC biosynthesized from other carbon sources. Currently, CME is not a valuable biomass output from cacao crops, and minimal applications have been reported for this material. Producing BC from residues, such as CME, will benefit the environment by cutting emissions of organic-rich effluents to surface water bodies, strengthening the cacao value chain in producer countries like Colombia by diversifying the cacao crop outputs while increasing materials circularity. Scaling-up the process is straightforward, and no significant changes in BC yields and production rates were observed between lab-scale and pilot-plant scale. This observation suggests a plausible industrial scenario for BC production from cacao fruits residual biomass.

2. Materials and methods

2.1. Cacao mucilage exudate (CME)

2.1.1. CME extraction and pretreatment

One-ton (1000 kg) of ripe cacao fruits from the CCN-51 (Colección Castro Naranjal 51) variety were harvested from a crop located in San Vicente de Chucurí, a small town nestled amidst a notorious cacao-producing region in the Department of Santander, Colombia. CCN-51 is a high-productivity cacao variety, resistant to common fungal diseases of the crop, extensively sown in cacao-producing countries. The cacao fruits were halved, the fresh beans extracted manually and placed in plastic sieves to collect fresh CME for 24 h. The liquid (labeled raw CME) was stored at −9 °C in airtight containers for further testing. The raw CME was subjected to heat-sterilization using an autoclave (Tuttnaver 3850 EL) at 120 °C and 125 psi for 20 min. The sample labeled sterilized CME, was also stored at −9 °C in airtight containers for further analysis and use in growth media formulation.

2.1.2. CME characterization

CME density at 20 °C was measured with a standard Gay-Lussac 5 mL pycnometer (BRAND® BLAUBRAND®) using water as reference material. pH at 20 °C was measured with a pH meter equipped with a glass electrode calibrated against buffer solutions of known hydrogen ion activity (METTLER-TOLEDO AG 8603 Schwerzenbach). Total solids were determined using the AOAC 932.12 method with a refractometer (Abbe 98.490 EUROMEX Microscopen B.V.). Moisture, ash, and protein percentages in CME were measured by gravimetric methods AOAC 931.04, AOAC 972.15, and NTC 1556 AOAC 970.22, respectively. Total nitrogen or Kjeldahl nitrogen (the sum of organic nitrogen and ammonia nitrogen) was determined with the 4500-N(Org) Semi-Micro-Kjeldahl Standard Method. Total phenolic compounds were measured via direct photometric readings (λ 500 nm) of a colored antipyrine complex formed by the reaction of steam-distillable phenols with 4-amino antipyrine in the presence of potassium ferricyanide (Standard Methods 5530 B and D). Individual and total sugar content analysis (saccharose, fructose, and glucose) was carried out with an Agilent technologies 1100 series HPLC liquid chromatograph equipped with automatic injection, an ion-exchange SUPELCOGEL C-610H (30 cm × 7.8 mm ID) column, and a refractive index detector (RID). The HPLC tests were performed using an injection volume of 20 μL of the sample, a 0.1% H_3PO_4 aqueous solution as a mobile phase with a flow rate of 0.5 mL/min, oven and RID temperatures of 30 °C and 35 °C, respectively, and total analysis time of 18 min per sample.

Trace metals quantitation (Na, K, Ca, Cd, Pb) was performed by atomic absorption, while Al was measured in emission mode, using a Thermo Solaar S4 instrument (Waltham, MA, USA). Raw and heat-sterilized CME were lyophilized in a LABCONCO lyophilizer at 0.080 mbar, and −87 °C for 72 h; the resultant solids were weighed and digested. For multielement (Ca, Na, K, and Al) and heavy metal (Cd and Pb) analyses, about 1.0 g of lyophilized raw or heat-sterilized CME were dissolved in 3 mL of concentrated nitric acid, stirred at 200 rpm and heated at 100 °C. After 2 h of reaction, 1 mL of concentrated perchloric acid was added to the mixture and heated at 100 °C. The digested sample was then transferred quantitatively to a 50 mL calibrated flask and diluted to the mark with de-ionized water.

2.2. BC production

2.2.1. Bacterial strain activation and seed culture (pre-inoculum) preparation

A freeze-dried sample of the bacterial strain *Gluconacetobacter xylinus* (ATCC® 23768™) was acquired from the American Type Culture Collection (ATCC, Manassas, VA, USA). Bacterial strain revival was carried out following the manufacturer's instructions. In short, the sample was stored in HS medium supplemented with 10% glycerol at −80 °C. Frozen *Gluconacetobacter xylinus* aliquots were reactivated in Hestrin-Schramm liquid medium (HS) (20 g L^{-1} glucose, 5 g L^{-1} peptone, 5 g L^{-1} yeast extract, 2.7 g L^{-1} Na_2HPO_4 and 1.15 g L^{-1} citric acid, pH 5.5), using an orbital shaker at 150 rpm, and 30 °C for 7 days (Hestrin & Schramm, 1954; Kurosumi et al., 2009). Seed cultures of *G. xylinus* were prepared using a microorganism suspension of 1×10^5 CFU mL^{-1} in a final volume of 150 mL of HS liquid medium, the mixture was incubated in an orbital shaker (JP Inglobal, Colombia) at 150 rpm, and 30 °C. The pre-inoculum was incubated for two days when the maximum biomass concentration was observed according to *G. xylinus* growth kinetics (E-Supplementary data).

2.2.2. BC production using Hestrin and Schramm and CME as culture media

A standard static fermentation set up for BC production consisted of 150 mL flask bioreactors containing 30 mL of one four different types of culture media tested. Three CME-based growth media were tested: raw sterilized cacao mucilage exudate (RCME), supplemented cacao mucilage exudate (SCME) (sterilized CME, 5 g L^{-1} peptone, 5 g L^{-1} yeast extract and 2.7 g L^{-1} sodium citrate), and diluted/supplemented cacao mucilage exudate (DSCME) (1:2 sterilized CME: water, 5 g L^{-1} peptone, 5 g L^{-1} yeast extract and 2.7 g L^{-1} sodium citrate). The Hestrin and Schramm (HS) liquid media (20 g L^{-1} glucose, 5 g L^{-1} peptone, 5 g L^{-1} yeast extract, 2.7 g L^{-1} Na_2HPO_4, and 1.15 g L^{-1} citric acid, pH 5.5) was used as a reference. Table S1 of the Supplementary Data shows media composition and BC productivity for various formulations, used to optimize bacterial cellulose production with the *Gluconacetobacter xylinus* strain ATCC®23,768™. The HS and the Yamanaka-ethanol (YME) media yielded the highest BC productivities (3,68 and 2,88 g L^{-1} respectively) with the ATCC®23768™ strain. For the SCME and DSCME media, the initial pH was adjusted to 5.5 using NaOH (3 N); however, during the fermentation, the pH was not controlled. For BC production, seed cultures of the reference strain *G. xylinus* (3 mL) were mixed with the culture media, and the flasks were placed in a dry incubator (Memmert IN750, Germany) at 30 °C for 15 days. All tests were carried out under sterile conditions.

Culture media samples and BC films were collected at 24 h intervals for 15 days to determine pH, sugar consumption, and BC content. For each culture media tested, we started with 45 fermentation bioreactors to account for triplicate experiments, and once the samples were collected, the three setups corresponding to each day were discarded. The total number of fermentations carried out was 180.

2.2.3. BC purification

After a fermentation period of 15 days, the BC membranes were removed from the culture media, immersed in boiling deionized water for 30 min, and then placed in an aqueous NaClO solution (5 wt%) for 72 h to remove remaining culture medium and adhered bacterial cells. The membranes were washed with distilled water to pH 7 and sterilized for 15 min at 121 °C (Yang et al., 2019). Finally, the purified BC films were lyophilized (LABCONCO lyophilizer) at 0.080 mbar, and −87 °C for 72 h. BC production was reported as g L^{-1} (dry mass (g) of BC/volume (L) of culture medium) (Gomes et al., 2013). Also, BC production yield, substrate conversion ratio, BC production rate, and BC production yield after 15 days of culture were calculated as follows:

$$Substrate\ conversion\ rate\ \alpha(\%) = \frac{Si - Sf}{Si} \times 100$$

$$BC\ production\ rate\ r_{BC}\ \left(g\ L^{-1}\ h^{-1}\right) = \frac{mBC}{V \times t}$$

$$BC\ production\ yield\ Y_{BC/S}(\%) = \frac{mBC/V}{Si - Sf} \times 100$$

where S_i is the initial substrate concentration (g L^{-1}), S_f is the final substrate concentration (g L^{-1}), *mBC* is the amount of BC produced (g), V is the reaction volume (L), and t is the culture time (h) (Gomes et al., 2013).

2.3. BC characterization

Cellulose functional group analysis was performed on a Bruker Tensor 27 Fourier Transform Infrared (IR-ATR) spectrometer fitted with a Platinum Diamond ATR unit A225/Q (Billerica, MA). Spectra were recorded at a resolution of 2 cm^{-1} in the range of 4000–500 cm^{-1}, a total of 32 scans were acquired for each spectrum. BC surface morphology was observed by field emission scanning electron microscopy (FESEM), using a FEI QUANTA FEG 650 (Oregon, USA) instrument fitted with secondary Everhart Thornley (ETD) and backscattered electron (BSED) detectors, and operated in high vacuum mode with an acceleration voltage of 15 kV. Due to the non-conductive nature of BC, samples were coated with a thin gold layer using a Quorum 150 T ES system (Oregon, USA). X-ray diffraction patterns of BC were measured by X-ray diffraction (XRD) spectroscopy on a Bruker D8 DISCOVER X-ray diffractometer (Billerica, MA) with a DaVinci geometry equipped with a CuKα1 radiation source (λ = 1.5406 Å, 40 kV and 40 mA), an area detector VANTEC-500, and a poly (methyl methacrylate) sample holder. Diffraction data were collected from 13.5 to 83.0 2θ degrees with a step size of 0.01. The peak-height method proposed by Segal was used to calculate the crystallinity index of cellulose:

$$CrI = \frac{I_{110} - I_{am}}{I_{110}}$$

where I_{110} is the height of the 1 1 0 peak representing both the crystalline and amorphous phases of bacterial cellulose, and I_{am} is the minimum between the 1 1 0 and 0 1 0 peaks representing only amorphous material (Segal et al., 1959). This method does not require background correction, so CrI calculations were made before background subtraction.

Also, the cellulose crystal size was calculated using the Scherrer equation, a mathematical relationship between the peak width of the diffraction peaks (FWHM, Full Width Half Maximum) and the crystal size:

$$\beta = \frac{K\lambda}{d\ Cos\ \Theta}$$

where *K* is a proportionality constant (0.9 assuming Gaussian profiles in XRD) is the X-ray wavelength and Θ is the Bragg angle. Finally, the thermal properties of freeze-dried BC films were measured on a TGA and DSC instrument STA 449 F5 JUPITER (NETZSCH, Gerätebau, Germany) under nitrogen atmosphere (20 mL/min). BC samples were placed on an Rh-Pt crucible and scanned from room temperature up to 500 °C at a heating rate of 10 °C/min.

3. Results and discussion

3.1. Cacao mucilage exudate (CME)

Fig. 1 shows the stages involved in CME production at a laboratory scale. The ripe cacao fruits are collected and halved (Fig. 1a and 1b), the

Fig. 1. Stages involved in CME production at a laboratory scale. (a) Cacao fruit, (b) open cacao fruits, (c) fresh cacao beans with mucilage, and (d) fresh and heat-sterilized CME.

fresh beans covered by mucilage (Fig. 1c) are manually extracted from the open pod and placed on plastic containers fitted with sieves at the bottom for simple CME collection. Fresh CME seeps from the cacao beans as a milky off-white fluid that transforms into a brownish/caramel liquid upon heat sterilization, as seen in Fig. 1d. Color change in CME, after sterilization, is attributed to the formation of colored species such as furfurals and caramelins resulting from thermal decomposition of sugars at high temperatures (Jiang et al., 2008). Approximately 4 $\pm$ 0.4 wt% of fresh CME was collected from one ton of fresh cacao fruit. The values reported correspond to an average of six measurements from different CME samples (fresh and sterilized) extracted from six different loads of cacao fruits (1 ton each) collected monthly from March to August 2019. CME is very similar, compositionally, to any other sweet fruit juice. The main components in CME are mono- and disaccharides (glucose, fructose, and saccharose) in concentrations ranging from 15 to 17°Brix for the fresh and heat-sterilized CME, respectively.

HPLC analysis allowed discrimination of sugars in CME. The total sugar content varies from 152.0 to 178.1 g L^{-1} for fresh and heat-sterilized CME, respectively. Fresh CME contains 60.60 and 80.68 g L^{-1} of fructose and glucose, respectively. Interestingly, the concentration of these two monosaccharides increases to 86.95 and 90.86 g L^{-1} in sterilized CME. On the other hand, the saccharose content, relatively high in fresh CME (10.71 g L^{-1}), is significantly reduced to 0.27 g L^{-1} in sterilized CME. We hypothesize that the increase in fructose and glucose, and the decrease in saccharose after heat-sterilization is the result of di- and oligosaccharides hydrolysis in fresh CME, promoted by the high acid content in the exudate (pH 3.77) and the high temperature of the process (121 °C).

The CME composition we report, in terms of sugar content and types, is similar to data found in the literature. For example, Balladares et al. reported 19°Brix and total sugar content of 12.33 wt% in *Theobroma cacao* L. sweatings (Balladares et al., 2016). The same authors found a higher fructose proportion in the samples (4.42 wt%), followed by glucose (2.15 wt%) and saccharose (2.13 wt%). Likewise, Anvoh et al. reported 16°Brix, 85% moisture, 3.76% ash, glucose, and saccharose content of 21.4 wt% and 2.13 wt%, respectively, in cocoa beans sweatings from South Côte D'Ivoire (Anvoh et al., 2009). These authors also found Ca, Na, and K contents of 171.5, 30.5, and 950 mg L^{-1} in the samples, respectively. Finally, Gyedu et al. report a percentage of total sugars of 7.5% in cocoa sweatings samples from Ghana (Gyedu, 2001). The concentration of saccharose, glucose, and fructose in cacao fruits may vary depending upon the age of the crop and the fruit ripeness, with immature pods containing higher proportions of saccharose. In contrast, ripe fruits are enriched mostly with fructose and glucose (Lefeber et al., 2010).

The chemical environment required for a microorganism to grow comprises mixtures of energy sources, minerals, vitamins, and nitrogen. These components are known as microbial growth factors (Kornmann et al., 2003). *G. xylinus* is a chemo-organotrophic organism that uses organic compounds as both energy and carbon source. Thus, for *G. xylinus* growth, the presence of simple and complex saccharides is of fundamental importance when considering an agro-industrial residue as alternative culture media. The presence of abundant saccharides in CME indicates a potential energy/carbon source for *G. xylinus* and the possibility of BC production.

In addition to sugars, an adequate growth media should contain sources of nitrogen and micronutrients; CME contains nitrogen (500 mg L^{-1}) and proteins (0.3%), as well as ash (0.4%). Nitrogen in CME may correspond to organic nitrogen in the form of amino acids. Balladares et al. reported the presence of amino acids, mainly glutamate (Glu) and aspartate (Asp), in cacao mucilage (Balladares et al., 2016). Interestingly, K in RCME is highly abundant, up to 2679 ppm, followed by Ca, Al, and Na with 59.88, 4.41, and 1.25 ppm, respectively. These results differ from reports by Afoakwa et al. related to cacao mucilage juice, particularly for Na (1330 ppm) and Ca (3160 ppm) (Afoakwa et al., 2013). Also, our findings are different from Anvoh et al.; these authors reported 1710 ppm of Ca, 300 ppm of Na, and 9500 ppm of K in cacao mucilage juice (Anvoh et al., 2009). The presence of trace elements in fruit juices and exudates is fundamental to promote cell growth and BC production by *G. xylinus* (Kurosumi et al., 2009; Som-ord et al., 2012; Stehlik-Tomas et al., 2004). These elements are key for the normal functioning of enzymes, generally as cofactors and transcription factors (Heo & Son, 2002; Stehlik-Tomas et al., 2004). For instance, $Mg2+$ and $Mn2+$ ions are necessary cofactors for the functioning of cellulose synthase and glucosyltransferases (Som-ord et al., 2012, Jonas & Farah, 1998; Vandamme et al., 1998). Also, $Na+$ and $K+$ contribute to maintaining the electrochemical gradient of solutes (Tortora et al., 2007; Yang et al., 2019). CME does not contain heavy metals such as Cd or Pb. Recently, the presence of Cd in cocoa beans has raised concerns due to the toxic nature of the metal and its bioaccumulation in human tissues (Huauya & Rofner, 2016).

Raw Cacao Mucilage Exudate (RCME) also has a relatively high and variable polyphenol content, with low values in the month of April (0.13 mg GAE/g RCME) to high values (1.7 mg GAE/g RCME) during January after the main harvest season (see Table S11). The average polyphenol content is 0.55 $\pm$ 0.45 mg GAE/g RCME. These polyphenols could effectively contribute to the production of BC compared to the reference culture medium HS. Polyphenolic compounds have been reported to stimulate bacterial cellulose production. Polyphenols can inhibit phosphodiesterases that degrade cyclic di-guanosine monophosphate (*c*-di-GMP), the most important regulatory factor in bacterial cellulose synthesis. Rani and Appaiah (2013) report positive effects of coffee cherry peel polyphenols (0.8%) on bacterial cellulose production (5.6 g L^{-1}). Also, Keshk and Sameshima (2006) report that polyphenols have antioxidant properties that can enhance BC production by decreasing the concentration of gluconic acid during fermentation.

3.2. Bacterial cellulose (BC) production with CME-based culture media

According to the compositional results presented in Table 1, CME is a sugar-rich residue with potential use as culture media for *G. xylinus* growth and BC biosynthesis. We started our tests using sterile CME as a culture medium without the addition of other nutrients or additives; the media was labeled Raw Cacao Mucilage Exudate (RCME). The RCME

Table 1
Physicochemical composition of CME from cacao fruits (var CCN-51).

Parameter	Raw CME	Heat-sterilized CME
	Mean ± SD	
Moisture (%)	84.71 ± 0.76	83.83 ± 0.78
Total dissolved solids (°Brix)	15.32 ± 1.33	17.55 ± 0.49
Total nitrogen (mg L^{-1})	518.47 ± 56.18	513.8 ± 76.13
Total phenols (mg L^{-1})	0.20 ± 0.15	0.21 ± 0.13
pH 20 (°C)	3.78 ± 0.09	3.77 ± 0.01
Density (g mL^{-1})	1.05 ± 0.02	1.07 ± 0.01
Protein (wt%)	0.29 ± 0.06	0.3 ± 0.02
Ash (wt%)	0.37 ± 0.10	0.40 ± 0.16
Total sugars (g L^{-1})	151.99 ± 8.87	178.08 ± 5.86
Glucose (g L^{-1})	60.60 ± 8.87	86.95 ± 5.86
Fructose (g L^{-1})	80.68 ± 3.35	90.86 ± 5.46
Saccharose (g L^{-1})	10.71 ± 4.13	0.27 ± 0.12
Calcium (mg kg^{-1})	59.88 ± 10.31	57.41 ± 8.58
Potassium (mg kg^{-1})	2679 ± 457.5	2698 ± 331.4
Sodium (mg kg^{-1})	1.25 ± 0.55	1.83 ± 1.08
Aluminum (mg kg^{-1})	4.41 ± 2.72	6.65 ± 1.18
Cadmium (mg kg^{-1})	N.D.[a]	N.D.
Lead (mg kg^{-1})	N.D.	N.D.

[a] N.D. Not detected.

media was inoculated with seed cultures of *G. xylinus* (10:1 culture medium:seed 1 × 10^5 CFU mL^{-1}). The performance of RCME as culture media was compared with the standard medium HS (20 g L^{-1} glucose, 5 g L^{-1} peptone, 5 g L^{-1} yeast extract, 2.7 g L^{-1} Na_2HPO_4, and 1.15 g L^{-1} citric acid, pH 5.5).

Fig, 2a and b shows the changes in pH, sugar concentration (g L^{-1}), and BC production (g L^{-1}) during fifteen days of fermentation with HS and RCME media, respectively. BC biosynthesis with RCME is low (0.55 ± 0.16 g L^{-1}) when compared with the conventional HS medium (4.20 ± 1.34 g L^{-1}). Despite the low value, BC biosynthesis with RCME proved to be similar or higher than reported yields for substrates such as juice and orange peel, 0.04–0.13 g L^{-1}, and apple, pineapple, Japanese pear, and grape juices, 0.2–0.4 g L^{-1} after 14 days of incubation (Kim et al., 2017). Thus, even in low quantities, the observation of BC films with the RCME medium demonstrates the feasibility of cellulose biosynthesis by *G. xylinus* using CME as culture media. However, we believe it is possible to boost BC productivity with CME by controlling critical compositional variables that may inhibit bacterial growth. For instance, the modest performance of RCME as a culture medium could be the result of compositional factors (individual or combined) such as pH, excess sugars, or nitrogen source availability.

The pH of RCME started at 3.35 and dropped to 2.59 during the process (Fig. 2b). Despite being an acidophilic organism, *G. xylinus* has optimal growth rates at pH values ranging from 4 to 6 (Jagannath et al., 2008; Mohammad et al., 2014). In contrast, the HS medium starts with a pH of 5.38 that drops to 3.5 after eight days of fermentation and then goes back at pH 5.36 at the end of the process (Fig. 2a). Many researchers report a decrease in pH in the culture media during fermentation, as a result of acetic and gluconic acid production by the microorganism (Lu et al., 2015; Revin et al., 2018; Vazques et al., 2013; Yang et al., 2019). Interestingly, when the medium is depleted of sugars, the microorganism can transform acids into Acetyl-CoA, which is metabolized into the tricarboxylic acid (TCA) cycle to generate ATP, a process that increases the pH of the medium (Revin et al., 2018; Souza et al., 2020). Also, BC production at pH above or below optimum values can be inefficient because the microorganism must use energy (ATP) to maintain intracellular level pH at the expense of cellular growth or BC production (Castro et al., 2011, Hwang et al., 1999; Jin et al., 2019). In terms of total sugar concentrations, RCME contains 158.26 ± 7.02 g L^{-1}, while the HS medium contains 20 g L^{-1} of glucose as a carbon source.

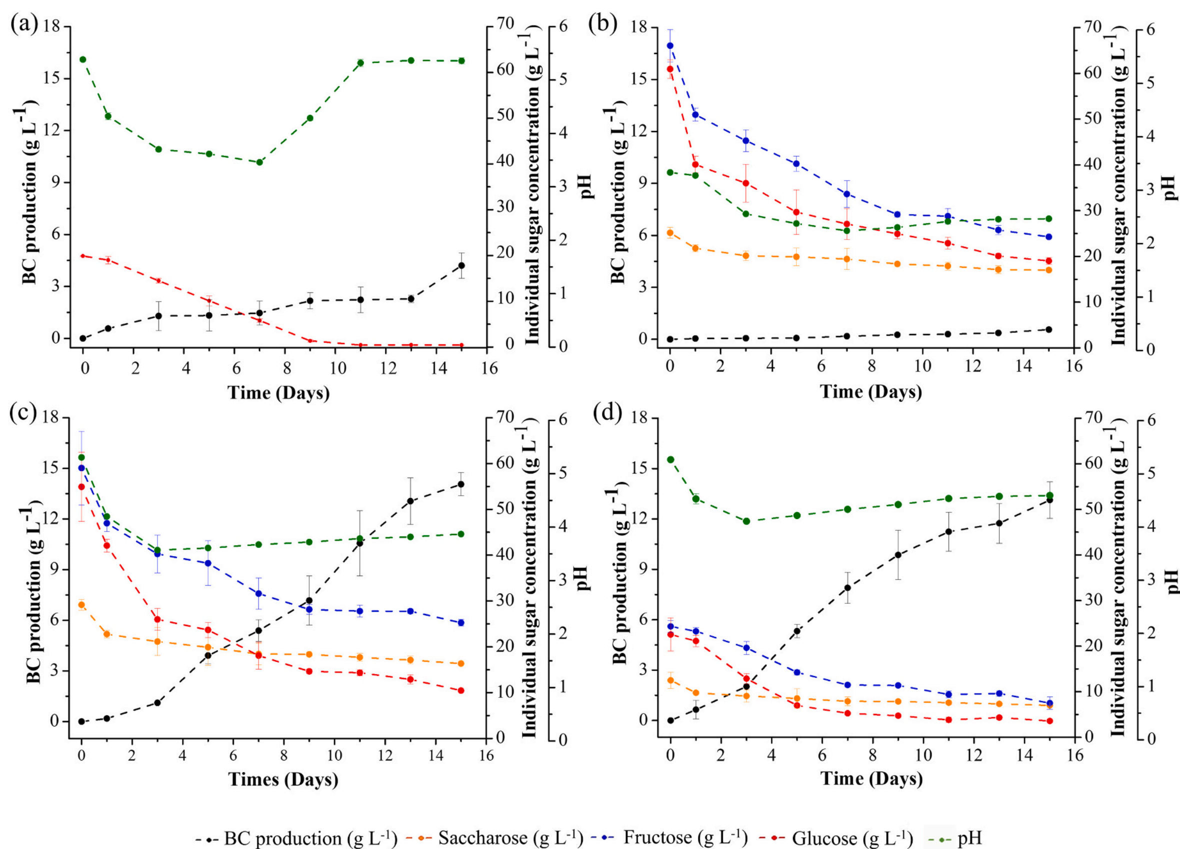


Fig. 2. Changes in pH, sugars concentration (g L^{-1}), and BC production (g L^{-1}) by *G. xylinus* during fifteen days of fermentation, using (a) HS, (b) RCME, (c) SCME, and (d) DSCME as culture media.

Several literature reports estimate total sugar concentrations ranging from 20 to 70 g L^{-1} for optimal *G. xylinus* growth. For instance, concentrations above 70 g L^{-1} of glucose, saccharose, or mannitol, and 30 g L^{-1} of glycerol, are reported to suppress bacterium growth (Chawla et al., 2009; Mohammadkazemi et al., 2015; Ramana et al., 2000; Yim et al., 2017). Thus, many bacterial genera exhibit a growth inhibition mechanism under high carbohydrate concentrations since the enzyme complexes responsible for transport into the cell can become saturated (Leite et al., 2019; Jin et al., 2019). Enzyme saturation by excess carbohydrates may explain why the high concentration of sugars in RCME might contribute to the limited production of BC with this medium. Also, excess glucose in the RCME medium could be converted to gluconic acid inhibiting cellulose production due to low pH (Revin et al., 2018).

In addition to sugars, a nitrogen source is vital for optimal *G. xylinus* development, and RCME contains ~0.3% proteins and ~500 mg L^{-1} of total nitrogen, as reported in Table 1. The HS medium, on the other hand, contains 5 g L^{-1} of yeast extract and 5 g L^{-1} of peptone as nitrogen sources. Although the nitrogen content in RCME, and perhaps its availability, are low, at this point is difficult to determine the impact of this variable on BC production. Nitrogen is essential for protein synthesis because the bacteria use it to form the amino group. It is also needed to synthesize DNA, RNA, and ATP (Mohammad et al., 2014). In most cases, when using alternative culture media, particularly agro-industrial residues, the use of N sources such as peptone, yeast extract, hydrolyzed casein, ammonium sulfate, or sodium glutamate always increases BC production in static fermentation processes with *G. xylinus* (Kurosumi et al., 2009; Pourramezan et al., 2009; Revin et al., 2018; Yang et al., 2019; Zeng et al., 2011). Therefore, to promote *G. xylinus* growth in CME, we formulated two new CME-based culture media. Initially, to assess the role of the nitrogen source availability, sterilized CME was supplemented with nitrogen sources in the form of yeast extract (5 g L^{-1}) and peptone (5 g L^{-1}). The resultant formulation was labeled supplemented cacao mucilage exudate (SCME). Secondly, CME was diluted with water (1:2 CME: H_2O) for a final total sugar concentration of 59.39 g L^{-1} (Fig. 2) and supplemented with yeast extract (5 g L^{-1}) and peptone (5 g L^{-1}). The resultant medium was labeled diluted/supplemented cacao mucilage exudate (DSCME) and was used to determine inhibition by excess carbon source. Also, both media were buffered with sodium citrate (2.7 g L^{-1}) to maintain pH values between 4 and 5.5 during the fermentation process.

Fig. 2c and d shows the changes in pH, sugar concentration, and BC production by *G. xylinus* during fifteen days of fermentation, using SCME and DSCME as culture media. The addition of a nitrogen source has a notable impact on *G. xylinus* growth. BC production increased thirty-fold, from 0.55 ± 0.16 g L^{-1} with RCME to 14.06 ± 0.69 g L^{-1} with SCME, only as a result of adding yeast extract (5 g L^{-1}) and peptone (5 g L^{-1}) to CME. Interestingly, with DSCEM, BC production also increased up to 13.13 ± 1.09 g L^{-1}, a value very similar to the one registered with SCME. Since these two media only differ in total sugar concentration (143.11 ± 8.03 for SCEM vs. 59.39 ± 8.72 g L^{-1} for DSCEM), and both produce the same amount of BC, we conclude that the limiting factor for *G. xylinus* growth in CME is not the sugar content, but rather the nitrogen source availability. BC production with the new formulated CME-based media (14.06 ± 0.69 and 13.13 ± 1.09 g L^{-1} for SCME and DSCME) is not only three times higher than with the reference medium HS (4.20 ± 1.34 g L^{-1} of BC), but also higher than with previously reported residues such as sugarcane molasses (4.01 g L^{-1}) (Bae & Shoda, 2008; Machado et al., 2018), corn liquor (6.9–12.8 g L^{-1}) (Noro et al., 2004), beer yeast (7.02 g L^{-1}) (Lin et al., 2014), coffee mucilage (4.80 g L^{-1}) (Florez, 2015), mango, guava, and Creole cherry extracts (12.67, 13.80 and 5.40 g L^{-1}, respectively) (Perna Manrique et al., 2018), maple syrup (1.50 g L^{-1}) (Zeng et al., 2011), grape, orange, apple, pear, and pineapple juices (2.10, 5.90, 3.90, 3.70 and 4.10 g L^{-1}, respectively) (Kurosumi et al., 2009), orange pulp, cane molasses, and tomato juice (2.8, 3.6 and 4.8 g L^{-1}, respectively) (Kumar et al., 2019), pineapple, pomegranate, muskmelon, watermelon, tomato, and orange juice, molasses, coconut water, and coconut milk (4.3, 3.8, 8.0, 6.1, 2.9, 6.2, 5.7, 6.2 and 3.9 g L^{-1}, respectively) (Hungund, 2013), Citrus peel (5.7 g L^{-1}) (Fan et al., 2016), potato peel wastes (1.27–2.61 g L^{-1}) (Abdelraof et al., 2019), carob and haricot beans (1.8 g L^{-1}) (Bilgi et al., 2016), and a mix of fermented fruits (plum, green grape, pineapple and apple) (6.40 g L^{-1}) (Jozala et al., 2015). Table S2 of the Supplementary Data compares BC production (g L^{-1}), yield (%), substrate conversion (%), and production rate (g L^{-1} h^{-1}) for cacao mucilage exudate (CME, this work) and various alternative residual biomass outputs reported in literature used to produce the biopolymer.

Additionally, Fig. 2 shows that the addition of sodium citrate to SCME and DSCEM helps with pH regulation during the fermentation process due to a buffer effect. CME contains citric acid (1–5 wt%), and the addition of the sodium salt of the acid helps form a citrate buffer with a pH range from 3.0 to 6.2. In SCME and DSCEM media, we observed pH values ranging between 4.0 and 4.5 during the process. Some authors report that supplementation with sodium citrate not only produces a buffering effect but also significantly decreases the production of acids by the microorganism (Chen et al., 2005; Li et al., 2012). Fig. 2 also shows that in the tested culture media, *G. xylinus* consumes glucose, preferably. For instance, for the RCME medium, the microorganism consumes 67% of the glucose present and only 33% of fructose. Likewise, for the SCME and DSCME media, glucose consumption reaches up to 81% and 84%, respectively, while fructose utilization corresponds to 57% and 69%, respectively. Saccharose decreases very little, between 10 and 15%, in the RCME, SCME, and DSCME media. In the standard HS medium, the microorganism consumed 98% of the glucose present, since it is the only energy/carbon source available. These results show that *G. xylinus* preferentially consumes glucose, which is directly converted to glucose-6-phosphate by phosphorylation in an oxidative phase that also produces NADPH (Donini et al., 2010). In the case of other sugars such as fructose, additional metabolic steps (that consume extra energy) are required to phosphorylate the sugar to produce fructose-6-phosphate, a compound that can be assimilated by the microorganism (Donini et al., 2010). Likewise, saccharose uptake by *G. xylinus* requires hydrolysis, an additional energy input, to produce glucose and fructose (Velasco-Bedrán & López-Isunza, 2007). When using complex sources of sugars (e.g., oligosaccharides or cellulose in residual biomass), several authors report a pretreatment involving sulfuric acid. Acid hydrolysis reduces the complexity of the carbon source to simple sugars easily assimilable by the microorganism for BC production (Bae & Shoda, 2008; Jaramillo et al., 2015).

In addition to growth factor analysis, operational parameters of the fermentation process are of fundamental importance when testing the performance of new culture media for a particular microorganism of biotechnological importance. Fig. 3 displays histograms showing the performance of the RCME, SCME, DSCME, and HS media in terms of substrate conversion (%), BC production rate (g L^{-1} h^{-1}), and yield (%) for BC production by *G xylinus*. There is abundant literature regarding operational parameters for BC production with alternative culture media, as seen in Table S2 of the Supplementary Data. To the extent of our knowledge, no scientific literature has been published, before our report, on the use of cacao mucilage exudate (CME) for BC production. Thus, according to Fig. 3, *G xylinus* reaches the highest substrate conversion (97.7%) in HS medium, followed by 68.6% and 62.7% in DSCME and SCME, respectively; the lowest conversion was observed with RCME (50.4%) (Fig. 3a). High substrate conversion values, up to 100%, are typical of culture media with monosaccharides as carbon sources. Interestingly, several authors also report high substrate conversion for alternative carbon sources such as glycerol and inositol (47% and 89%), orange, apple, pear and pineapple juices (69%, 39%, 48%, and 39%, respectively), cheese whey (42%) and mixed sources from agro-industrial residues like cashew apple juice and soybean molasses (55% and 37%) as seen in Table S2 (Carreira et al., 2011; Keshk & Sameshima, 2005; Kurosumi et al., 2009; Mikkelsen et al., 2009; Souza et al., 2020). Also, using SCME and DSCME as culture media, *G. xylinus* reached

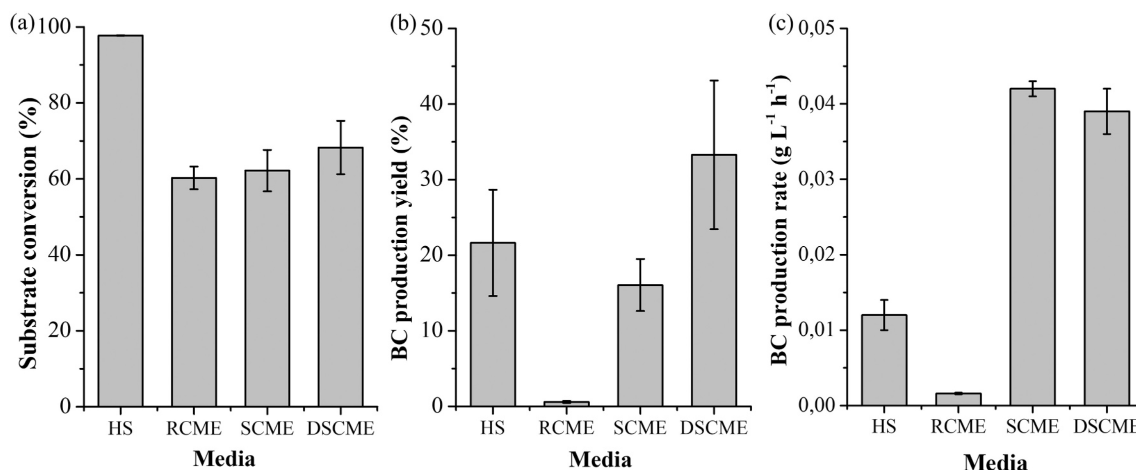


Fig. 3. (a) Substrate conversion, (b) BC production yield, and (c) BC production rate for cellulose biosynthesis by *G. xylinus* using HS, RCME, SCME, and DSCME as culture media, after 15 days of fermentation.

maximum productivity rates of 0.0390 and 0.0365 g L^{-1} h^{-1} of BC, respectively. In contrast, the productivity rates of BC were lower with HS (0.0093 g L^{-1} h^{-1}) and RCME (0.0012 g L^{-1} h^{-1}), respectively. The use of diluted and supplemented CME allowed an increase of more than 400% in the productivity rate in contrast with the reference medium HS, and more than 3250% to the unmodified RCME.

For instance, when using crude glycerol as a carbon source with *Gluconacetobacter xylinus* a BC rate of 0.039 g L^{-1} h^{-1} was reported (Yang et al., 2019), also, productivity rates of 0.0013, 0.005, 0.004, 0.021, 0.016 and 0.028 g L^{-1} h^{-1} for BC biosynthesis were registered with grape juice, grape skin extract and cheese whey, sugar cane molasses, orange, and tomato juice, respectively, as seen in Table S2 (Carreira et al., 2011; Kumar et al., 2019). Concerning the BC yield, a term that correlates BC production (g L^{-1}) to available sugars in the culture medium (g L^{-1}), the highest value was measured with DSCME, 33%, followed by the HS medium, 27.5%. The lowest yield of BC 0.6% was measured with the RCEM medium, which may be due to the low pH of the medium and the absence of a nitrogen source in proportions adequate for BC synthesis (Fig. 3b). Also, it should be taken into account that glucose was the only substrate in the HS medium (20 g L^{-1}), while

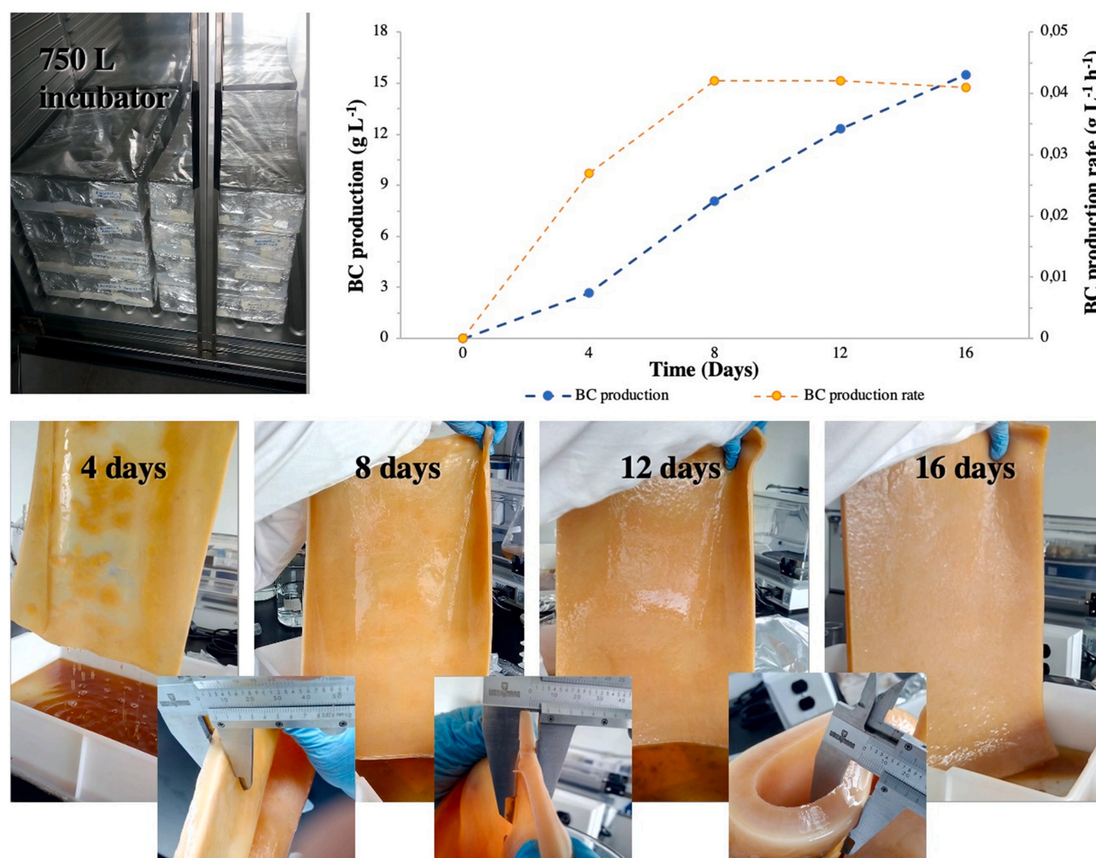


Fig. 4. BC production scale-up to 15 L reactors with a working load of 3 L of growth medium (DSCME). Top right, BC production yield, and BC production rate for cellulose biosynthesis by *G. xylinus* during fifteen days of fermentation. The bottom images show the resulting BC (film thickness in the inset).

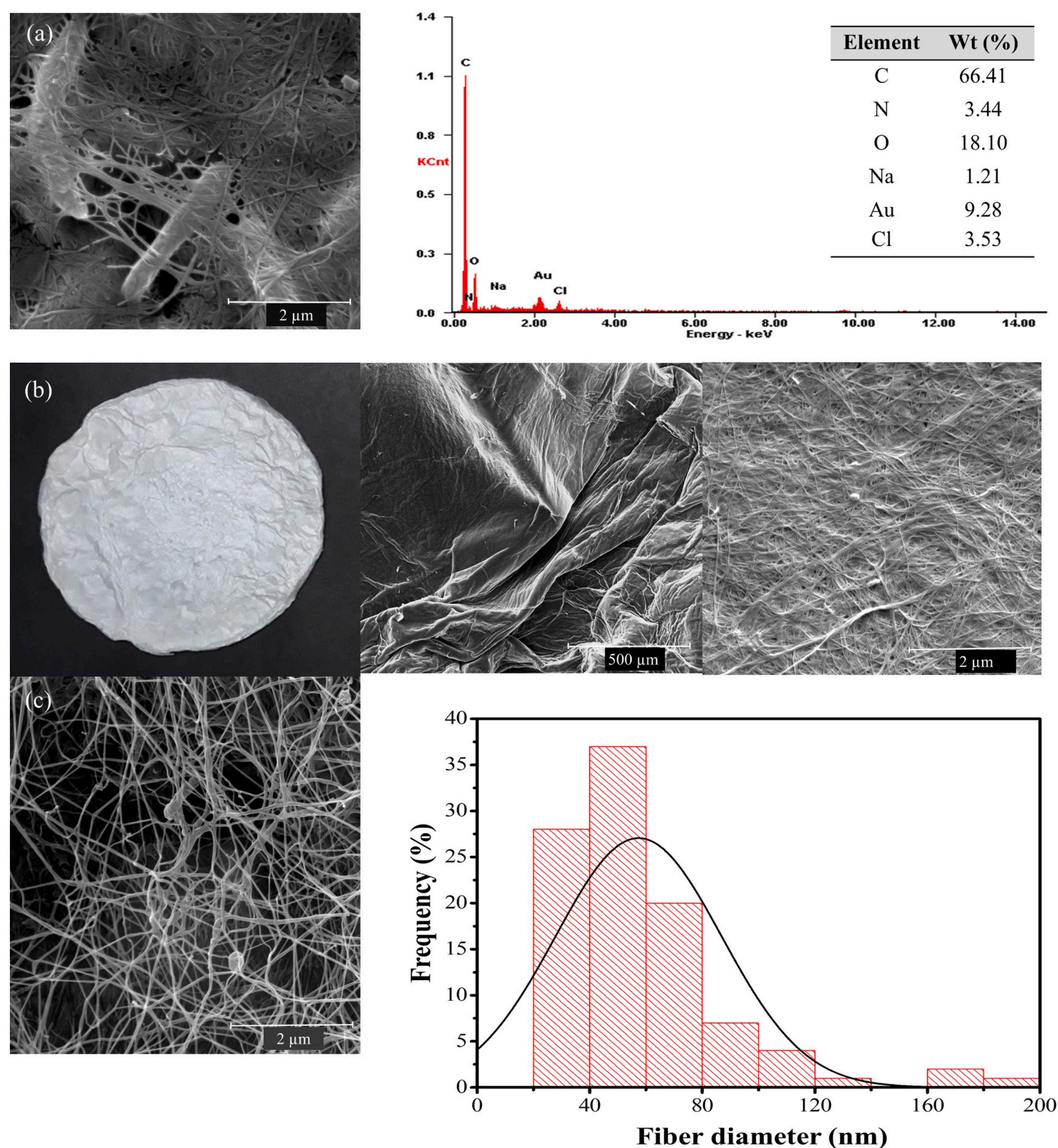
other sugars were present in the CME-based media.

As discussed in the preceding sections, a maximum yield of 13.13 ± 1.09 g $L^{-1}$of BC was reached when using diluted and supplemented CME (DSCME) in fermentation reactions carried out in 30 mL glass reactors. With these conditions as starting point, we scaled up BC production in a batch process using 15 L high-density polypropylene rectangular reactors (L: 52 cm, H: 9 cm, W: 33 cm) with a working load of 3 L of growth medium (DSCME). The process was monitored for 16 days and carried out in a 750 L incubator (Memmert IN750, Germany) with a capacity for forty reactors (15 L each), as illustrated in Fig. 4. The BC yield in these reactors was 519,33 g L^{-1} of wet BC (15,57 g L^{-1} dry BC) with a production rate of 0,041 g L^{-1} h^{-1}, as seen in Fig. 4 (top right). BC production remains relatively similar for both assemblies (30 mL and 15 L). Thus, we believe the scaling-up process using batch reactors is a viable alternative for BC production using CME.

From the perspective of an industrial scale process, each tonne (1000 kg) of cacao fruits yields an average production of 40 L of CME. The growth media with the best performance -DSCME- has a composition of 1:2 sterilized CME: water, 5 g L^{-1} peptone, 5 g L^{-1} yeast extract, and 2.7 g L^{-1} sodium citrate; thus, the theoretical volume of growth media (DSCME) that can be formulated per tonne of cacao fruit is 120 L. Fermenting 120 L of DSCME to produce BC would require 40 rectangular batch reactors with the dimensions specified above. We are currently working on a financial model for BC production using CME.

3.3. BC film characterization

Using simple sugars in CME *G. xylinus* is able to produce cellulose as a compact and robust tridimensional network of intertwined nanofibers. Fig. 5 shows optical and SEM images of BC films produced from diluted and supplemented cacao mucilage exudate (DSCME) as culture media for *G. xylinus* growth. After the BC film is removed from the culture media, we observe *G. xylinus* cells still attached to the lower side of the film (cellulose/culture medium interface), Fig. 5a. The elemental composition analysis shows carbon and oxygen as the most abundant elements in the BC film, followed by nitrogen from *G. xylinus* cells, and



Element	Wt (%)
C	66.41
N	3.44
O	18.10
Na	1.21
Au	9.28
Cl	3.53

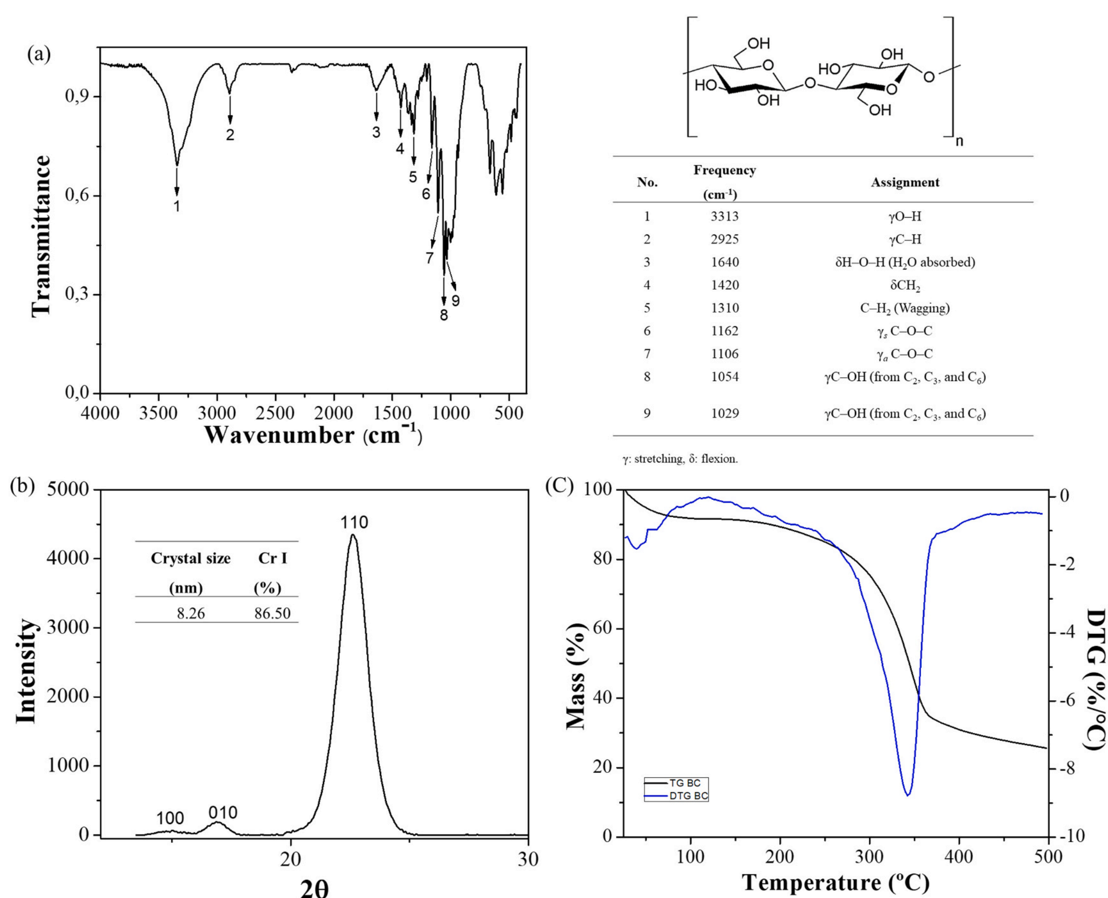
Fig. 5. Optical images and SEM micrographs of BC films produced by *G. xylinus* using diluted and supplemented CME. (a) SEM of raw BC showing *G. xylinus* attached to the network and elemental composition of the film, (b) optical image of lyophilized BC film after treatment with NaClO, and SEM images of the BC surface, (c) mechanical dispersion of the treated BC film showing the fiber diameter distribution.

gold from the coating used to process the sample for SEM analysis. Fig. 5b shows a white BC film after treatment with NaClO to remove adhered *G. xylinus* cells and the caramel-brown color that the film acquires by contact with the culture medium. Although NaClO is not commonly used to treat bacterial cellulose membranes, it can effectively remove the color from the culture medium and eliminate non-cellulosic materials such as *G. xylinus* cellular debris from the BC films. In 2011 Gea et al. reported that NaClO does not alter BC crystal structure and improves the material's mechanical properties. In contrast, NaOH does not eliminate color effectively and at concentrations higher than 6% induces structural changes in cellulose from type I to type II (Gea et al., 2011).

SEM images of the lyophilized films show the crumpled surface morphology, the network of individual nanofibers, and the pores in the film resulting from the random organization of the nanofibrils. These small orifices give the BC films the ability to absorb and retain water, an important characteristic of the material (Mikkelsen et al., 2009; Yim et al., 2017). Fig. 5c shows the cellulosic fibers with average width distribution between 50 and 60 nm and micrometric lengths. Literature reports fibrils widths ranging from 10 to 100 nm for BC (Moosavi-Nasab & Yousefi, 2011; Nguyen et al., 2008; Yim et al., 2017). Several authors report morphologically similar structures in studies using conventional carbon sources (glucose, fructose, and saccharose) (Mikkelsen et al., 2009; Mohammadkazemi et al., 2015; Yim et al., 2017), as well as agro-industrial wastes (Carreira et al., 2011; Kurosumi et al., 2009; Li et al., 2015).

Fig. 6a shows the IR spectrum with characteristic signals corresponding to cellulose, as previously reported by other authors (El-Saied et al., 2008; Z. Li et al., 2015; Moosavi-Nasab & Yousefi, 2011; Nguyen et al., 2008; Souza et al., 2020). A broad band in the region between 3313 and 3341 cm^{-1} corresponds to the stretching of free and bonded (inter- and intramolecular) hydroxyl groups (-OH) abundant in cellulose. The in-plane C-O-H bending modes are observed at 1428, 1362, and 1272 cm^{-1}, while the signals at 1054, 1029 cm^{-1} correspond to C–OH stretching modes (from C2, C3, and C6) (Salari et al., 2019). The characteristic C-H antisymmetric and symmetric stretching frequencies of methylene groups show at 2925 and 2893 cm^{-1}, respectively. Also, the CH_2 bending and wagging modes are observed at ~1410–1420 cm^{-1} and 1310 cm^{-1}, respectively (Shahabi-Ghahfarrokhi et al., 2015). Regarding characteristic signals for the pyranose ring of the anhydroglucose unit of cellulose, the strong bands at 1162 cm^{-1} and 1106 cm^{-1} correspond, respectively, to the antisymmetric and symmetric stretching of the C-O-C bridge and the ring antisymmetric in-phase stretching mode. Also, the anomeric carbon C1-H exhibits a characteristic signal at 947 cm^{-1}. Finally, the band at 1630–1638 cm^{-1} arises from vibrations of water adsorbed on the amorphous regions of cellulose, or it can also be attributed to the stretching vibration of the (γ) –CO-NH– moiety, which could originate from residual bacterial cells and proteins in BC after the cleaning treatment (Santos et al., 2015).

Fig. 6b shows the X-ray diffraction profile of the BC after treatment with NaClO. Information regarding the type of cellulose, crystallinity index, and packing arrangement can be extracted from XRD. Native or natural cellulose, known as cellulose polymorph type I, is produced by living organisms as a mixture of two crystalline forms, I_a and I_b. The two crystalline forms may coexist; however, cellulose in bacteria and algae is enriched in the Iα form, while cellulose in higher plants and animals (tunicates) is essentially Iβ. The I_a crystalline allomorph of cellulose is arranged in a triclinic unit cell containing one chain, whereas the I_b is a monoclinic crystal with two chains. Fig. 6b shows three characteristic signals of native cellulose. The peak at 14.27° 2θ corresponds to



No.	Frequency (cm^{-1})	Assignment
1	3313	γO–H
2	2925	γC–H
3	1640	δH–O–H (H_2O absorbed)
4	1420	$δCH_2$
5	1310	C–H_2 (Wagging)
6	1162	$γ_s$ C–O–C
7	1106	$γ_a$ C–O–C
8	1054	γC–OH (from C_2, C_3, and C_6)
9	1029	γC–OH (from C_2, C_3, and C_6)

γ: stretching, δ: flexion.

Fig. 6. (a) FT-IR spectrum, (b) XRD pattern, and (c) thermograms (TGA/DTG) of a BC film produced by *G. xylinus* using diluted and supplemented CME as culture medium.

overlapping crystallographic planes (010)I_a and (100)I_b, whereas the signal at 16.73° 2θ rises from crystallographic planes (100)I_a, (110)I_b (French, 2014; French & Santiago Cintrón, 2013; Santos et al., 2015). At 22.52° 2θ we observe contributions from crystallographic planes (110)I_a and (200)I_b. The crystallographic data reported match with the cellulose I profile recorded at the International Center for Diffraction Data (JCPDS), file No. 50-2241 (Czaja et al., 2004).

Depending on their origin and treatment, cellulosic materials have different crystallite sizes (Czaja et al., 2004). The Scherrer equation is widely used for the estimation of cellulose crystal size because of its independence of crystallite shape and symmetry. Using the Segal method and the Scherrer equation, we determined the crystallinity index (CI) and cellulose crystal size, as seen in Fig. 6b. BC films produced by *G. xylinus* using DSCME as culture media exhibit a crystallinity index (CI) of 86.5%, and crystal size of 8.26 nm corresponding to approximately 21.15 chains of cellulose considering the (110) direction of the I_a polymorph (French & Santiago Cintrón, 2013). The observed CI is within range of those of other BC films obtained from carbon sources such as glucose and glycerol (CI: 78 and 85%, respectively) (Jung et al., 2010), a mix of fermented fruits (plum, green grape, pineapple, and apple) (CI: 70%) (Jozala et al., 2015), sugars from processed rice bark (CI: 58%) (Goelzer et al., 2009), dry olive mill residue (CI: 80%) (Gomes et al., 2013), cashew apple juice and soybean molasses (CI: 79 and 80%, respectively) (Souza et al., 2020), and glycerol from biodiesel production (CI: 74%) (Yang et al., 2019). Also, the crystal size is similar to values reported for BC films produced by *G. xylinus* (Ruan et al., 2016). Interestingly, some authors also mention that in cellulose samples, increasing crystallite sizes translate into a concomitant reduction of the amorphous regions and an increase of CI. The crystal size in BC, 8.26 nm, is larger than crystal sizes in plant-derived cellulosic materials ranging from 3 to 7 nm (Hindeleh & Johnson, 1972; U. J. Kim et al., 2010; Poletto et al., 2014).

Finally, TGA and DTG curves in Fig. 6c account for the thermal behavior of the BC film. Thermal degradation of cellulose occurs through an irreversible set of endothermic reactions such as dehydration, depolymerization, oxidation, and decarboxylation. The first thermal event at 100 °C corresponds to a weight loss of 7.87% attributed to the evaporation of residual water (Mohite & Patil, 2014). The second event, with a weight loss of 64.05%, occurs between 150 and 400 °C and is attributed to cellulose depolymerization to yield levoglucosan. The process involves the initial breakdown of the main polymer structure to produce oligosaccharides, which further transform into levoglucosan (LGA). Dehydration and isomerization reactions of LGA result in anhydrosugars formation that undergo dehydration, fragmentation, and condensation reactions to form furfural-derivatives, hydroxyacetone, glycolaldehyde, and glyceraldehyde, among other compounds. Above 450 °C, low molecular weight cellulose decomposition products can either undergo polymerization to form char or turn into CO and CO_2 via decarbonylation and decarboxylation reactions (Lin et al., 2009; Zhang et al., 2010).

Finally, we compared the structural characteristics of the BC membranes produced with CME with BC films obtained from other residual biomass, as seen in Table S3 of the Supplementary data. Regardless of the growth media composition, the BC crystal structure always corresponds to native cellulose (type I). The Crystallinity Index of BC varies widely among BC from several sources, ranging from 65 to 90%, with BC from CME exhibiting high crystallinity (87%). Crystal size in BC from CME (8.26 nm) is similar to BC obtained from pecan nutshell and distillery effluents. The nanofibers morphology also varies broadly, with widths ranging from 10 to 190 nm, and BC from CME exhibiting average widths between 50 and 60 nm. The thermal degradation temperature of BC from several sources ranges from 340 to 384 °C with BC from CME degrading at 350 °C.

4. Conclusions

CME is a nutrient-rich agro-industrial by-product from cacao beans production. Bacterial cellulose films can be readily biosynthesized by *G. xylinus* growing directly on CME. However, the high sugar content, low pH, and limited nitrogen sources of the CME affect BC yields by hindering *G. xylinus* growth. Formulation is required to use CME as culture media for *G. xylinus* growth and bacterial cellulose biosynthesis. Dilution (1:2 CME: H_2O) and the addition of a nitrogen source can effectively improve the performance of CME as culture media and boost BC production. Diluted and supplemented CME (DSCME) afforded BC yields of 13.13 ± 1.09 g L^{-1}, in contrast to 4.20 ± 1.34 g L^{-1} with the standard HS, and 0.55 ± 0.16 g L^{-1} with -raw- RCME. BC biosynthesis rate with DSCME was 0.0365 ± 0.0030 g L^{-1} h^{-1}, while with HS was 0.0093 ± 0.0037 g L^{-1} h^{-1} and 0.0012 g L^{-1} h^{-1} with RCME. The bacterial cellulose yields with CME-based culture media are high, when compared with media composed of other residual effluents from agro-industrial processes reported in literature. Interestingly, although the substrate conversion rate with the DSCME media only reached 68.23 ± 7.03%, further dilution of CME did not increase the substrate conversion rate. Thus, the spent DSCME media was successfully tested as culture media for the production of bioplastics, which will be the topic of an upcoming contribution. Finally, using the DSCME as growth media, BC production was scaled up from 30 mL to 15 L reactors using a 750 L incubator with capacity for 40 reactors in static culture mode. The maximum BC yields and production rates observed at lab-scale (13.13 g L^{-1} and 0.0365 g L^{-1} h^{-1}) were replicated at pilot-plant scale (15,57 g L^{-1} and 0,041 g L^{-1} h^{-1}). This fact suggests an industrial scenario where BC production from cacao mucilage exudate, a residual biomass from cacao fruits processing, is feasible.

CRediT authorship contribution statement

Olga L. Saavedra-Sanabria: Investigation, Methodology, Validation, Visualization, Formal analysis, Writing – original draft. **Daniel Durán:** Investigation, Methodology, Validation, Visualization, Writing – original draft. **Jessica Cabezas:** Investigation, Methodology, Validation, Visualization, Writing – original draft. **Inés Hernández:** Conceptualization, Formal analysis, Supervision, Project administration, Data curation, Writing – original draft. **Cristian Blanco-Tirado:** Conceptualization, Resources, Funding acquisition. **Marianny Y. Combariza:** Conceptualization, Formal analysis, Supervision, Writing – review & editing, Funding acquisition.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.carbpol.2021.118645.

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