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AGRICULTURAL WASTES AS A CARBON OR NITROGEN SOURCE FOR PRODUCTION OF BACTERIAL CELLULOSE. A MINI REVIEW

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ABSTRACT

Bacterial cellulose (BC), a biopolymer, has gained importance in the recent past due to its physicochemical properties, which are desirable for various biotechnology, microbiological, and material science applications. Since cost is a significant limitation in the production of cellulose, there have been efforts to identify potential means of addressing this issue. Currently, all efforts are focused on using agricultural waste as a cost-effective substrate for the synthesis of microbial cellulose. This is to solve an existing environmental problem with the accumulation of these wastes and the resulting damages such as environmental pollution. Uncertainties abound regarding the capacity for large-scale commercial production of microbial cellulose using different types of waste materials. This study investigates researches on the feasibility of using waste as a source of carbon and nitrogen for commercial-scale production of bacterial cellulose. Preliminary findings reveal the potential to yield a high concentration of bacterial cellulose from various agricultural wastes. Moreover, recent research activities in the production of BC are also discussed. This review, at the same time, discusses some applications of BC briefly. The findings indicate an agricultural waste is an effective and good source for cellulose production.

KEY WORDS : Bacterial cellulose, Agricultural waste, Microbial cellulose, Carbon source, Nitrogen source.

INTRODUCTION

Bacterial Cellulose (BC) continues to attract scholarly attention, perhaps due to its unique properties, such as biodegradability and biocompatibility, a high degree of purity, and ease of polymerization (Stanislawska *et al.,* 2020). These properties make BC one of the materials with a broad array of applications. Although BC offers many beneficial properties, the relatively expensive production process limits its application. Despite its numerous benefits, the high cost of BC production limits its industrial implementation and the market share of polymers. A standard medium used to produce BC is Hestrin–Schrammian, an expensive medium composed of glucose, yeast, peptone, citric acid, and potassium (Hestrin and Schramm, 1954). As the widespread use of BC depends on a significant reduction of its production cost. There is a need to identify a cost-effective and sustainable carbon source to produce BC that does not compete with food production. In recent years, many researchers have discussed using common and abundant agricultural wastes as a carbon source to reduce the significant production costs of BC.

Reviewing and analyzing the literature can reveal essential developments in the field and facilitate the assessment of future practicality of this substantial production of BC for various industrial applications. This paper examines the feasibility of using agricultural waste in BC production through an extensive review of the literature to underpin the

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current state of knowledge and identify discernible trends and gaps inexperience. Since various wastes contain high carbon and nitrogen content, their utilization as substrates could yield high concentrations of microbial cellulose, albeit with optimization of culture conditions. In this manner, the high-value utilization of agricultural waste is deemed economically, environmentally, and pragmatically advantageous.

Overview of Bacterial Cellulose

Bacterial cellulose (commonly known as bio-cellulose), which is the purest form of cellulose, continues to receive widespread attention due to its superior physicochemical properties in comparison with plant cellulose, in which impurities like hemicellulose and lignin are often found (Picheth *et al.,* 2017). In contrast, BC has unique properties, which include high tensile strength, crystallinity, water-holding capacity (WHC), as well as slow water release rate (WRR) and remarkable mold ability into three-dimensional structures (Wang *et al.* 2020). BC is produced as extrusions of glucose chains from the bacterial body via small pores present on their cell envelope. These extrusions are responsible for forming micro-fibrils that further aggregate into web-shaped cellulose ribbons networks with many empty spaces between the fibers. The well-separated nan fibrils of BC create an enlarged surface area and highly porous matrix. The basic fibril structure contains a β-1→4 glucan chain with the molecular formula $(C_6H_{10}O_5)n$ and is held together by hydrogen bonds. These micro-fibrils are almost 100-fold smaller than the fibrils of vegetal cellulose (Costa *et al.,* 2017).

Production of bacterial cellulose

The production of BC has been extensively reviewed (Güzel and Akpinar, 2019; Moniri *et al.,* 2017; Ul-Islam *et al.,* 2020; Volova *et al.,* 2018). Various aerobic, non-pathogenic bacteria produce bacterial cellulose from the genera *Gluconacetobacter*, *Acetobacter*, *Sarcina*, *Rhizobium*, and *Agrobacterium* in both synthetic and non-synthetic media (Ullah *et al.,* 2019). However, these bacteria do not possess the photosynthetic capacity and therefore need glucose or some organic substrate synthesized by a photosynthetic organism to assemble their cellulose (Keshk, 2014). The main obstacles encountered in the applications of BC and its up-scale production are the high cost of the media and low productivity at the industrial scale. The viable possibility to address the production cost issue is to exploit the renewable carbon sources through bioprocess optimization, thus, reducing the usage of costly commercial media and low productivity (Jang *et al.,* 2017). Converting wastes and by-products to BC by the microbes on an industrial scale will help to attain one of the UN's sustainable development goals of ''responsible production and consumption'' (Akinsemolu, 2020). scientists are currently making use of natural/waste resources with enhanced biological synthesis approaches to get significant products to develop a ''zero waste'' society and economy (Romano *et al.,* 2019). In recent times, a worldwide campaign for the development of innovative technologies for the transformation of industrial wastes into valuable materials has been launched. One such approach is the "clean-biotechnology that makes use of fermentation to recycle and reuse tons of low-cost substrates and wastes from various industries (agro, food, brewery) to produce BC, the production of which involves fermentation in either static or agitated conditions.

The most commonly used cultivation medium for cellulose producing bacteria is a chemically-defined medium known as the Hestrin–Schramm (HS) medium. This medium requires relatively expensive additional components such as yeast extract, glucose, peptone, disodium phosphate, and citric acid, which ultimately leads to high production costs. Modification of growth conditions including pH, temperature, and carbon source and its concentration affects both the quality and quantity of BC produced. Besides, different cultivation methods result in the production of BC with varying structures and properties (Blanco *et al.,* 2020; Numata *et al.,* 2019).

The volume of air supply and concentration of the carbon sources determine the yield of microbial cellulose under static conditions. Insufficient oxygen supply can render bacteria inactive, which is a significant limitation in statistic production environments. Agitated conditions can produce higher yields, but the mechanism of BC formation remains unclear under different production conditions (Chen *et al.,* 2019; Vazquez *et al.,* 2013).

More extensive applications of BC would be dependent on practical considerations concerning scale-up capability and production costs. Many studies have focused on different strains of cellulose-producing microorganisms, cheap nutrient sources, and additional components to produce value-effective BC (Blanco *et al.,* 2020; Hussain *et al.,* 2019;

Ul-Islam *et al.,* 2020). Numerous waste products from different sources, such as industrial waste, whey, agro-industrial, and wastewater, have been investigated as potential substrates for improved BC production.

Microbial Cellulose from Agricultural Waste

Agricultural waste is considered of economic and cultural significance worldwide. It is a potential and abundant biomass source of renewable energy, convertible to environmentally friendly nature, readily available, low cost, and potentially sustainable. Agricultural activity produces tons of biomass daily and just 10% of it is used as alternative raw materials for industries (Hasan and Ammenberg, 2019). The use of agro-biomass for BC production will lower the cost significantly and help enhance its sustainability, as well as make it "green" and marketable. Acetic acid pre-hydrolysis liquor of agricultural corn stalk was used as a cheap carbon source for the green synthesis of BC. Several studies have examined the viability of using various sources of agro-waste for BC production. For instance, Lima *et al.* (2017) used sisal juice as a substrate to produce BC, using *Komagataeibacter hansenii* (Lima *et al.,* 2017). The researchers evaluated the effects of various variables on the production potential, including the pH, sugar concentration, nitrogen supplementation, and duration of cultivation. The findings showed that the best possible BC yield from sisal waste was 3.38 g/L, which took 10 days of bacterial cultivation at pH 5. The study suggests that sisal waste is a valuable resource for producing BC, but there are concerns about the availability of sisal waste for extensive manufacturing. There have been attempts to assess the potential of other agricultural wastes as carbon sources for BC production, including coffee cherry husk (CCH), mango pulp (MP), citrus peels (CP), potato peels (PP), banana peels (BP), pineapple, orange peels (OP), and date fruits waste (DFW).

CCH was used as a potential agro-industrial substrate for BC production, which is a by-product present in abundance from coffee cherry processing (Rani and Appaiah, 2013). Results of research attempting to produce BC from CCH show that the production capacity of up to 8.2 g/L can be achieved using 8% CCH extract in combination with steep corn liquor, under optimized conditions. Since CCH is an abundant agro-industrial waste, there have been attempts to use this waste material as a substrate to produce BC (Rani & Appaiah 2013). The results show a production capacity of up to 8.2 g/L of BC using 8% CCH extract combined with steep corn liquor, under optimized conditions. The findings agree with research evidence that steep corn liquor is a rich nutritional source that can supply organic content, such as carbon and nitrogen (Joshi *et al.,* 2018).

Mango is considered one of the most important tropical fruits globally; it is ranked among the top 10 fruits globally in terms of the total production of major fruit crops. Approximately 30%-50% of the mango fruit goes into the waste, leading to around 200,000 lakh tons of waste generated each season by the pulp industries in these areas, which are being discarded without proper treatment methods. MP could provide an essential substrate for BC production. Similar results were obtained using mango and guava purees (Viana *et al.,* 2018). García-Sánchez (2020) used MP and yeast as a nitrogen source by *Komagataeibacter xylinus*. The researchers found the production of BC (6.32 g/L) was obtained after 16 days in static culture (García-Sánchez *et al.* 2020). According to the researchers, the product's water vapor permeability increases significantly with the addition of fruit puree. Other modifications in the produced BC include a reduction in tensile strength and enhanced elongation.

Citrus peels (CP), not consumed with fruits due to their bitter taste, make up nearly 30–60 g/100 g of the citrus fruit weights. Güzel and Akpinar (2019), used CP (lemon, mandarin, orange, and grapefruit) to produce BC. They found the BC yields were between 2.06 and 3.92 when using *Komagataeibacter hansenii* GA2016 (Güzel and Akpinar 2019). Another study by Tsouko *et al.* (2020), used orange peels as a carbon source for BC production by *Komagataeibacter sucrofermentans*. They found 11.6 g/L BC concentration with a yield of 1.55 g/L/day in tray bioreactors under air sparging (Tsouko *et al.,* 2020). Another study by Kuo *et al.* (2017) compared the traditional (HS) medium with CP using *Gluconacetobacter xylinus*.

They found BC yield to be 4.2–6.32 times higher than in the conventional (HS) medium (Kuo *et al.,* 2017). The same study found the production of BC from CP medium was 5.7 ± 0.7 g/L, which was higher than from HS medium (3.9 ± 0.6 g/L) (Fan *et al.,* 2016).

Potato peel waste (PPW) is considered a "zero value waste," which is found in sizeable quantities following industrial potato processing and can range from 15 - 40% of original product mass, due to the peeling method. Yearly, large amounts of PPW remain from industrial potato processing. Untreated PPW is not suitable for non-ruminants as it is too fibrous for digestion. Still, it is a relatively inexpensive waste and includes a large volume of starch, non-starch polysaccharides, lignin, polyphenols, protein, and a small number of lipids. Different researchers agree with research evidence that PPW is a rich nutritional source, which can supply organic content, such as carbon and nitrogen. In light of all these properties, PPW is viewed as a low-cost and precious base for fermentation processes. Abdelraof *et al*. (2019) used PPW for BC production by *Gluconacetobacter xylinus*. They found the highest BC yield (4.7 g/l) was noted after six days at 35 °C (Abdelraof *et al.,* 2019).

Banana peels (BP) are another agricultural waste with the potential to provide the required carbon. Large amounts of BP waste are produced from juice industries and fruit markets daily. These BP wastes are a potential bio-resource, but if unused and dumped in landfills will represent an environment risk (Sial *et al.,* 2019). It can be converted into useful material instead of dropped in landfill sites, reducing the environmental and economic problems. To solve this problem, some researchers have used BP for BC production. Research efforts to produce BC from banana peels as carbon source using *Acetobacter xylinum* showed the ability to provide 19.46 g/l of the product, with a cultivation period of 15 days and temperature of 30 ºC. Using BPW to produce BC would be economic value and benefits. It would also eliminate the environmental risk of pollution if the agro-waste were to be discarded without prior treatment. In the case of coconut water and pineapple juice as a substrate for *Acetobacter. xylinumm* similar results were obtained (Sunun *et al.,* 2014). Moukamnerd *et al.* (2020) made efforts to produce BC from BP as a carbon source using *Komagataeibacter nataicola*. The result showed the ability to produce BC was 13.85±1.18 g/l with a cultivation period of 9 days (Moukamnerd *et al.* 2020). One such study showed that using *Acetobacter xylinum* up to 19.46 g/l of BC could be produced in a cultivation period of 15 days at 30 ºC (Sunun *et al.* 2014).

Pineapple peels (PP) constitute the largest share of by-products production (30% and 42%). Generally, half of the total pineapple weight represents both a by-product and a potential source of useful compounds (Roda and Lambri, 2019). To achieve the most efficient reduction in waste production and minimize environmental pollution, there is a need to determine and characterize the waste produced. Pineapple fruit has been noted to be an enriched raw material with insoluble fibers, pectin's, simple sugars, and proteins as the major compounds. There is also a decent level of micronutrients, including vitamins, minerals, and phenolic compounds (Roda and Lambri, 2019). The sugars composition of pineapple waste has been analyzed by Sepulveda *et al.,* (2018), who noted 82% of total sugars, reducing (55%) and non-reducing (27%), respectively (Sepúlveda *et al.,* 2018). They contained high sugars in the waste that can be used as a good carbon or nitrogen source for BC (Kumbhar *et al.,* 2015). In a related study, Castro *et al.* (2011) used PP for BC production using *Gluconacetobacter swingsii*. The comparisons were based on HS medium as the reference standards. Thy found that PP juice could produce up to 2.8 g/l of BC, which was higher than the reference standards (2.1g/l) (Castro *et al.,* 2011).

Another fruit used for BC production was date fruit. The date fruit is another potential source of carbon. Dates grow mainly in tropical and arid regions of North Africa and Southwest Asia. Date syrup contains essential nutrients considered adequate for the growth of various microorganisms (Moosavi-Nasab and Yousefi, 2010). However, the processing of dates is accompanied by massive loss and wastage, which can be converted to useful by-products. Lotfiman *et al.* (2018) assessed the feasibility of producing BC from date syrup as a carbon source using *A. xylinum* (Lotfiman *et al.* 2018). The researchers identified sugar content in the waste sample using HPLC and tested different concentrations of the fruit syrup obtained using different culture times. BC production was achieved with a 3% (w/v) date in the media cultivated for eight days. Modification of the HS medium led to an increased BC yield of up to 68%. It was concluded that date waste could be a useful source of carbon. Other cellulosic wastes from non-food wastes have also been used for BC formation, albeit with reduced BC yields, such as the use of olive mill residues, which produced 0.81 g/l of BC (Gomes *et al.,* 2013). This BC yield was relatively low compared to the yield from date syrup. All this indicates that agricultural waste could be a rich source of carbon compared to non-food sources.

Besides the substrates, as mentioned above, waste from the date industry is another potential substrate for BC's low-cost production. One such by-product of this industry is date syrup (DS), which is rich in carbohydrates (Al-Mssallem *et al.,* 2020). In a study utilizing low-quality DS, which has very little commercial value, Moosavi-Nasab and Yousefi (2011) found that with the use of DS, BC production showed a steady increase up to day 14 of cultivation, compared to sucrose. At the end of agriculture, cellulose yields using DS (4.35 g/l) were more than two-folds that of sucrose (1.69 g/l). It was attributed to the presence of abundant reducing sugars in DS, compared to sucrose, which is a disaccharide (Moosavi-Nasab and Yousefi, 2010). Another group of researchers used the same substrate, Lotfiman *et al.* (2018), to evaluate BC production by *A. xylinum*. Their study indicated that the microorganism could produce up to 5.8 g/L of BC, which is 68% higher than that of the standard HS medium (Lotfiman *et al.,* 2018).

This section has provided a comprehensive roundup of various researchers' work in their experiments producing BC using various agro wastes ranging from mango peels to banana, and pineapple peels to date fruit syrup and even coconut water. The significance of all these experiments is the transformation of what would have been discarded agricultural waste into economically valuable BC and in the process contribute to the reduction of environmental pollution which would have been caused by these same agro wastes if they were discarded in landfills without prior treatment/ The following section logically disuses research pertaining to the applications of BC in various industrial domains.

Applications of BC

The exclusive and excellent physical and mechanical properties of BC make it the most suitable material for use in a variety of fields. Currently, BC has used cellulose as a novel substitute polymer in several applications, which adds more advantageous attributes to making end products superior to standard substitutes (Hussain *et al.,* 2019). The unique properties of BC were the cause of its widespread use. Humans have benefited from this material for centuries, as so many cellulose-derived applications have been developed. BC products are essential in our lives, such as biomedical, food, cosmetics, vascular grafting, filtration, electrical and sensor applications, energy production, paper production, and the textile industry (Hussain *et al.,* 2019).

In 1986, used BC in biomedical applications by purified ethylene oxide sheets were successfully used as temporary skin replacements in 40 hospital patients in Brazil (Fontana *et al.,* 1990). On the other hand, other medical applications under investigation include flat BC membranes, interference meshes and cartilage replacement tissue substitutes (Pang *et al.,* 2020), nasal septa (Mandour *et al.* 2019), vocal folds (Bodea *et al.,* 2020), dura mater (Binnetoglu *et al.,* 2020), corneal (Zhang *et al.* 2020).

BC is a food fiber approved by the Food and Drug Administration (FDA) (Shi *et al.,* 2014). One of the main advantages of BC as a food-based ingredient is the use of food based on human indigestion (Willerth and Sakiyama-Elbert, 2019). In addition to contributing to the oral sensation, it promotes intestinal transit (and other dietary fibers) (Fontana *et al.,* 2017). Nata-de-coco (Ngan *et al.* 2019), rheological booster (Huang *et al.,* 2019), fat substitutes (Vigentini et al. 2019), organic food (Li *et al.,* 2020); Pickering Emulsion Stabilizer (Mitbumrung *et al.,* 2019), and probiotic and enzyme immobilizer are the main applications of BC for the food industry (Azeredo *et al.,* 2019). The characteristics and structure of BC as a food ingredient make it possible to avoid flavor interactions and increase food stability by extending the pH range, temperature, and freezing conditions (Shi *et al.,* 2014). Other potential uses of BC in the food industry include gelling, thickening, emulsifying, stabilizing, and water-binding agents (Blanco *et al.,* 2020).

It was also suggested that BC should be used as an environmentally friendly filter material. Many components have recently been added to BC to increase selectivity and reduce molecular weight. BC membranes have been used to supply a porous network that may contain graphene oxide (GO). The resulting BC-GO composites showed both advanced properties, water stability, good mechanical strength, and selective ion permeation up to the GO scale. These benefits make the BC-GO a promising resource for the purification of the water and drug industries (Fang *et al.,* 2016).

The BC can be modified and applied to electrical applications. Functional BC has produced a conductive NP silicone and polyaniline BC network capable of retaining its flexibility and other physical properties. This material could have been a promising anode for Li-ion batteries (Park *et al.* 2016). BC has also been used to summarize cobalt ferrite nanotubes that can be used as nanowires for

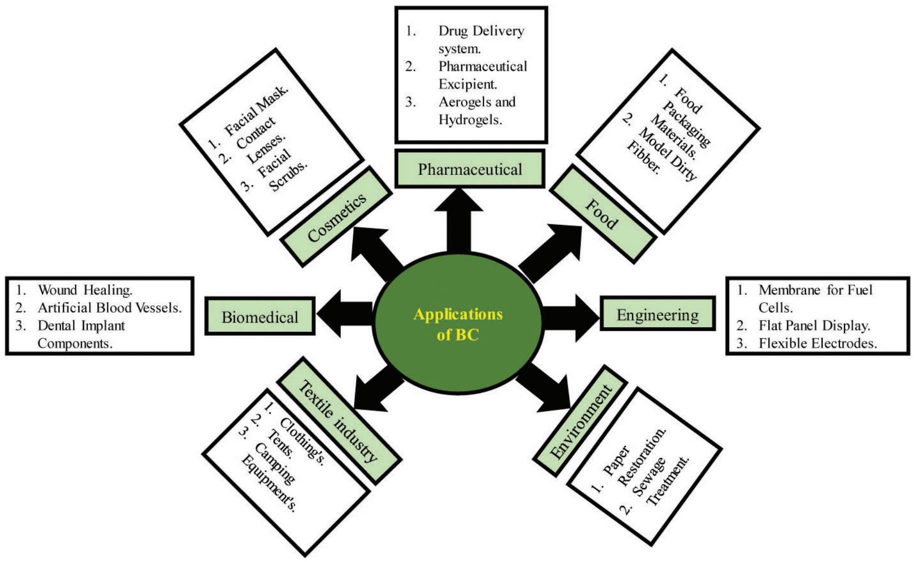

Fig. 1. Some of the industrial applications of BC

a variety of electronics (Menchaca *et al.,* 2016). Sustainable development to protect the environment has remained in place. Metabolic systems engineers are still working to integrate biomass into sustainable bio-based bio-ethanol production systems (Jang *et al.,* 2017). BC has an excellent capacity to achieve this goal. The BC-produced cellulose film will serve as a green and cost-effective substitute for bioethanol for sustainable fermentation. Moreover, since BC does not produce lignin and hemicellulose, the pretreatment step may be omitted in industrial processes to reduce production costs.

BC also assessed the manufacture of paper as a high-quality paper additive, increasing its current demand for market characteristics such as tensile indexes or tear indexes with reduced porosity and elongation (Fillat *et al.,* 2018).

Most of these applications (summary in Fig 1) have been used for the production of BC by *Gluconobacter*. *Gluconobacter* is the genus of choice for research and scale-up in the industry due to the higher BC yield (Ruka *et al.,* 2012). With the increasing use and development of technology, the demand for bacterial cellulose is steadily increasing. As a result, efforts have been made to establish a low-cost and efficient BC production method with high yield and improved properties.

CONCLUSION

Bacterial cellulose, due to its unique structural features, offers desirable properties, is considered a desirable biomaterial for various applications across many fields. The study findings show the technical and economic feasibility of producing microbial cellulose from wastes. The overarching conclusion is that "most of the agricultural wastes have the potential to produce high concentrations of bacterial cellulose. It is especially possible with the optimization of bacterial culture conditions, such as temperature and pH. More importantly, the findings demonstrate that the produced microbial cellulose would have desirable chemical, physical, and mechanical properties that suit various applications. Overall, large-scale commercial production and demand of microbial cellulose using waste as a carbon and energy source can lower the biomaterial production cost and help eliminate or reduce the economic and environmental burden of industrial waste.

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