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BACTERIAL CELLULOSE PRODUCTION FROM AGRO-INDUSTRIAL WASTEWATER FOLLOWED BY BIOGAS PRODUCTION: TECHNICAL FEASIBILITY OF IMPLEMENTING A BIOREFINERY CONCEPT

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ABSTRACT

Bacterial cellulose (BC) was produced by *Gluconacetobacter hansenii* grown statically at room temperature. The BC media were the same as the Hestrin and Schramm medium, with only the original carbon source (glucose) replaced with agro-industrial residues: glycerol; vinasse or 50% vinasse + 50% whey. After 28 days of growth, the glycerol medium achieved the highest BC per surface cultivation area (4.09 mg/cm^2) and productivity (0.146 mg/cm^2/d). A search of the Scopus database revealed a lack of publications on the treatment of effluent from BC production. Therefore, this paper detailed a ground-breaking process in which glycerol was employed as a raw material for BC production and its effluent (BCE) was processed in an anaerobic sequencing batch reactor (ASBR) to produce biogas. Anaerobic digestion of BCE was carried out in bioreactors containing granular biomass operated at 100 rpm, 30 °C, and 24-hour cycle length. At 2.0 g-COD/L, BCE treatment resulted in 77% COD removal efficiency and 2.79 mmol-CH_4/L. Experimental data using glycerol allowed estimating the maximum potential for BC production on an industrial-scale, obtaining 63 tons-BC/month from 5,800 tons-glycerol/year generated by a Brazilian biodiesel industry. The ASBRs required for industrial biogas production from BCE treatment would be 2.8×10^4 m^3, and the methane produced could be burned to generate 3.2×10^2 kW.

Keywords: Agro-industrial residues. Anaerobic digestion. Bacterial cellulose. Biogas. Glycerol.

1 INTRODUCTION

Bacterial cellulose (BC) has high potential for diverse applications, but its low productivity and high production costs prevent widespread use. Recently, many studies have been conducted seeking the development of low-cost fermentation media for its production, encouraging the use of residues and by-products as substrates for the process, which can improve BC production's competitiveness and economic feasibility[1]. In this way, agro-industrial wastewaters have the potential to be used as substrates for BC production, which would lower production costs. However, the problem of environmental adequacy of these residues would still be unsolved, due to the large amount of wastewater generated and the organic matter remaining even after this process. A solution to this issue would be the production of biogas (methane) from the anaerobic treatment of BC effluent (BCE), which could be used in boilers to produce steam and electricity. Thus, there would be environmental adequacy of these wastewaters and energy production. Therefore, this work studied the laboratory and industrial feasibility of the integrated production of biomolecules (BC) and bioenergy (biogas) from agro-industrial wastewaters (glycerol, vinasse, or whey), contributing to a circular economy and fitting into the biorefinery concept.

2 MATERIAL & METHODS

Microorganism: *Gluconacetobacter hansenii* (ATCC 23769) – Collection of Tropical Cultures (CCT) of the André Tosello Research and Technology Foundation, São Paulo, Brazil – and preserved in test tubes containing inclined Mannitol solid culture medium in a refrigerator (4 °C)[2].

Bioreactor: Assays were conducted in 500 mL Erlenmeyer flasks with a surface area of 70.88 cm², containing 100 mL of culture medium and inoculated with 5.0% (v/v) *G hansenii* suspension.

Culture media: The composition of the different culture media used in BC was: Standard culture[3]; Hestrin and Schramm[4]; and Modified Hestrin and Schramm, which the difference between the original and modified Hestrin and Schramm medium is the residues used as carbon sources – bidistilled glycerol from a Brazilian biodiesel production industry, vinasse from a sugar and alcohol plant in the Brazilian state of São Paulo, and powdered industrial whey.

BC production: Table 1 presents the conditions for static BC cultivation performed in batch mode at room temperature (around 25 °C). After removing the BC films, samples of the residual culture medium were taken to determine the concentrations of organic matter (in terms of COD), carbohydrates, glycerol, total volatile acids, and bicarbonate alkalinity according to the Standard Methods for the Examination of Water and Wastewater[5].

Performance indicators: The performance indicators used for bacterial cellulose production were the mass of BC produced per surface area of cultivation (MCA – mg/cm^2) and productivity (Pr – mg/cm^2/d), defined as the MCA divided by the number of cultivation days.

Table 1 Conditions for BC growing in all assays.

Conditions	Culture medium	Carbon source	C_{S0L} (g-Substrate/L)	t (d)
1	HS	Glucose	20	10/20/28
2	MHS	G	20	10/20/28
3	MHS	G	40	10/20/28
4	MHS	V	20	10/28
5	MHS	V/W	20	28

Notation: C_{S0L}: Initial substrate concentration; t: Cultivation time; HS: Hestrin and Schramm; MHS: Modified Hestrin and Schramm; G: Glycerol; V: Vinasse; W: Whey; V/W: 50% vinasse + 50% whey.

Using the laboratory-scale BC production data, the most promising agro-industrial residue, among those evaluated, for a scale-up feasibility study was defined as the one that achieved the best BC performance indicators. The first step of this assessment is to determine the annual output of this residue on an industrial-scale. With this value and laboratory data, the maximum potential BC production on full-scale could be estimated, admitting the utilization of all surplus waste generation from a biorefinery as substrate.

The methodology proposed by Lovato et al.[6] was utilized to evaluate energy production via anaerobic treatment of BCE and glycerol separately on an industrial-scale. Applying this methodology to BCE required determining the monthly volume (V_{I-IND}) to be treated in an ASBR for biogas production and the COD concentration. The choice of a reactor with operating mode in sequencing batches was justified because bacterial cellulose is produced in batches. Details of the operation of such a reactor can be found in the article by Selma et al.[7].

3 RESULTS & DISCUSSION

Table 2 presents the results obtained for BC production under all studied conditions.

Table 2 Results obtained for BC production.

Conditions	Carbon source	C_{S0L} (g-Substrate/L)	t (d)	MCA (mg/cm^2)	Productivity (mg/cm^2/d)
1	HS	20	10/20/28	1.03/1.99/2.02	0.103/0.099/0.072
2	G	20	10/20/28	1.18/1.96/4.09	0.118/0.098/0.146
3	G	40	10/20/28	2.58/3.96/5.16	0.258/0.198/0.184
4	V	20	10/28	0.37/0.81	0.037/0.029
5	V(50%)/W(50%)	20	28	0.73	0.024

Notation: C_{S0L}: Initial substrate concentration; t: Cultivation time; MCA: Mass of BC produced per surface area of cultivation; HS: Hestrin and Schramm; G: Glycerol; V: Vinasse; W: Whey.

As seen in Table 2, MCA increased from 1.03 to 1.99 g/cm^2 after 10 and 20 days of cultivation in Condition 1, respectively. This increase was not reflected in yield values (0.103 and 0.099 mg/cm^2/d), indicating a downward trend in this parameter with the increase in cultivation days. At 28 days of cultivation, the increase in MCA was irrelevant, causing a drop in productivity and reinforcing the tendency. Conversely, the glycerol-based culture medium (Conditions 2 and 3) was promising, as the glycerol medium was more conducive to BC formation than the standard HS medium, whose primary carbon source is glucose. Comparing the results of Conditions 1 (HS – 20 g/L) and 2 (Glycerol – 20 g/L) revealed that with 10 and 28 days of cultivation, the increase in productivity was approximately 14 and 202%, respectively. Also, the increase in the glycerol concentration (from 20 g/L in Condition 2 to 40 g/L in Condition 3) resulted in an increase in the membrane mass obtained, with the highest value observed in Condition 3 (5.16 g/cm^2), for a 28-day culture period. However, the highest productivity was obtained after 10 days of cultivation in a glycerol medium at a concentration of 40 g/L (0.258 mg/cm^2/d). Vinasse culture medium presented the lowest MCA and BC productivity regardless of the cultivation time compared with Conditions 1 to 3. Condition 5 evaluated whether the combination of substrates (whey and vinasse) would favour BC production and it was revealed that it was not favourable. Glycerol was the culture medium chosen to further investigate biogas generation, since Conditions 2 and 3 achieved the highest MCA and productivity.

The results obtained for BC production from a glycerol-based culture medium were used for scale-up estimation, as this agro-industrial residue, among those studied, presented the highest performance indicators for BC production. For processing 5.8×10^3 tons/year of glycerol, 4.6×10^7 reactors would be necessary, achieving a maximum of 63 tons-BC/month.

The estimated BC mass (M_{BCmax}) is the maximum that might be achieved by directing all surplus glycerol demand into BC production. It may not be feasible to allocate the entirety of this by-product to BC manufacture. However, these results indicate that the process can fulfil the growing demand for BC. The suggested process could be adjusted to the desired BC demand, requiring 728 reactors per kilogram of BC produced monthly. The major limitation of this process is the applicability of BC production on an industrial-scale due to the requirement to maintain the same geometry properties (superficial area and equipment height) to guarantee a culture medium depth between 1.1 and 4.5 cm since, at greater depths, the production is lower due to oxygen limitation in the surface area of the culture medium[8]. Therefore, future studies could contribute to developing new technologies and configurations that optimize BC production systems and facilitate the scale-up.

The monthly volume of culture medium produced from glycerol destined for industrial-scale BC production was 1.4×10^7 L/month. Considering an average water loss of 55%, the influent flow rate of the wastewater from the BC production process ($V_{(I-IND)}$) is

6.2×10^6 L/month. The concentration of BCE entering an industrial ASBR was 47.7 g-COD/L. Thus, to maintain laboratory conditions (2.0 g-COD/L), dilution would occur inside the reactor due to residual volume. The results show that the methane production capacity of the methanogenic reactor fed with BCE and glycerol was 3.5×10^4 and 8.9×10^4 mol-CH_4/d, respectively. Thus, the single AD system produces more CH_4 than the combined BC and methane production system. However, the first system is more compact than the second one since it needs to process less organic matter, which leads to similar methane productivity for both systems (1.24 and 1.14 mol-CH_4/m^3/d for BCE and glycerol treatment, respectively). The BC production process and subsequent treatment by anaerobic digestion is advantageous because it could obtain two high-value products: BC and methane. Energy generated can supply industry demand or be sold. This result contributes to the advancement of feasibility studies for implementing the concept of biorefinery in the soybean industry (in which one of the by-products is glycerol), as it shows the possibility of using by-products (such as glycerol) to produce BC, energy, and liquid effluent from the AD reactor as fertilizer. Figure 1 depicts the flowchart of both scenarios studied.

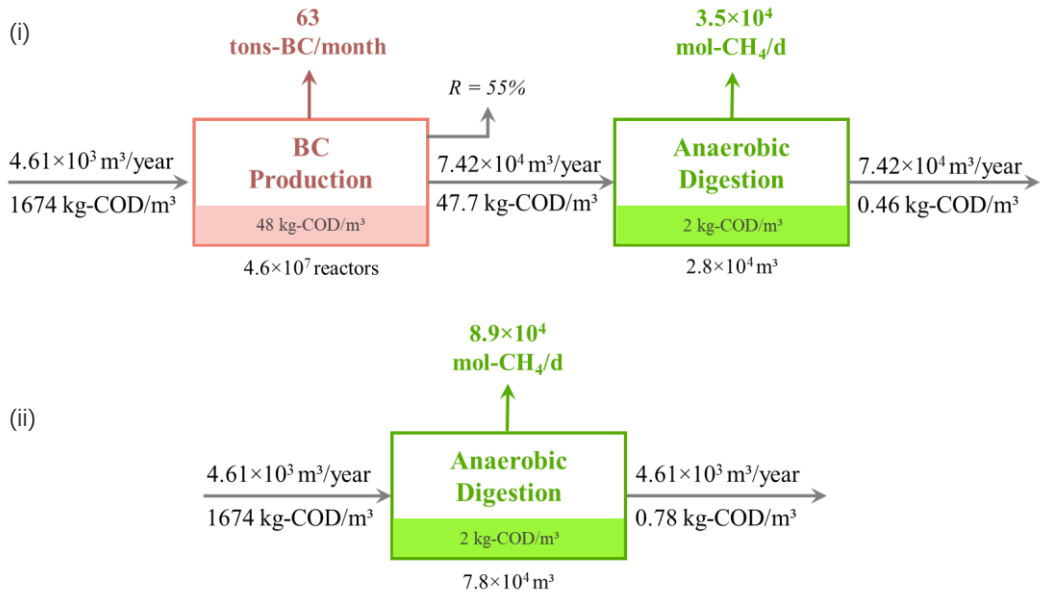

Figure 1 Flowchart of the comparison scenarios of (i) BC production using glycerol + AD of BCE; and (ii) AD using glycerol.

4 CONCLUSION

Using several substrates for bacterial cellulose production revealed that glycerol was a viable carbon source capable of achieving higher MCA and productivity than standard Hestrin and Schramm medium, vinasse, and 50% vinasse + 50% whey. BC production applying glycerol as a substrate generates a large volume of effluent with a high concentration (47.7 g-COD/L), which justifies the treatment of this effluent (BCE) in an anaerobic reactor to reduce its polluting potential and to generate biogas (methane). At 2.0 g- COD/L, the anaerobic digestion of the BCE achieved 77% COD removal efficiency, 2.79 mmol-CH_4/L, and 3.63 mol- CH_4/kg-COD. Estimating the industrial production potential of bacterial cellulose using all the surplus glycerol produced by a Brazilian industry (5,800 tons/year), a maximum production of 63 tons/month would be possible. Treating all BCE generated in an anaerobic sequencing batch reactor (ASBR) for biogas recovery could generate 3.2×10^2 kW, obtaining a residual digestate with approximately 0.5 g-COD/L.

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