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Techno-economic feasibility assessment of bacterial cellulose biofilm production during the Kombucha fermentation process

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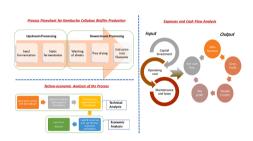
HIGHLIGHTS

- Kombucha cellulose biofilm (KCB) fermentation depends on operational conditions.
- Techno-economic analysis (TEA) of production and processing of KCB.
- Facility dependent and labor charges are key economic drivers.
- KCB production is economically feasible with reasonable rate of returns.
- Payback time of 4.23 years was predicted through TEA.

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GRAPHICAL ABSTRACT



ABSTRACT

Bacterial cellulose produced during Kombucha fermentation has recently received lots of attention owing to its desirable mechanical and physicochemical properties and is exploited for different food, textiles and environmental applications. However, lack of information on process feasibility often hinders large-scale manufacturing of Kombucha-based cellulose. Therefore, the current study assesses techno-economic feasibility of a 60-ton annual capacity Kombucha-based cellulose production facility using *SuperPro* designer. Economic feasibility analysis showed an estimation of 13.72 million US\$ as total investment and 3.8 million US\$ as operating costs with 89% expenses associated with facility dependent and labour costs. The process feasibility is revealed with a payback time of 4.23 years, 23.64% return on investment and 16.48% internal rate of return. Sensitivity analysis presented that increased volume of fermentation units and automating the process can significantly reduce input costs. Such research is necessary to aid policymakers in facilitating the commercialization of Kombucha-based cellulose at field scale

1. Introduction

Cellulose is the most abundant polymer on earth, approximately contributing to 1.5 trillion tons of total biomass annually, especially from plant-based sources like hemp, cotton etc. and also from microbial sources like bacteria or algae (Moniri et al., 2017). Bacterial cellulose

(BC) is a naturally occurring biopolymer mostly produced from a mixed consortium of acetic acid bacteria belonging to *Acetobacter* and *Gluconobacter* species and osmophilic yeast species like *Zygosaccharomyces* sp., *Saccharomyces* sp., and *Schizosaccharomyces* sp. (Azeredo et al., 2019). In contrast to plant-based cellulose which is difficult to process due to the presence of lignin and hemicellulose, BC does not contain these

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components, therefore it need not to be treated via delignification and detoxification and can be processed with ease via mild alkali treatment (Klemm et al., 2001; Laavanya et al., 2021). BC based biofilms have a highly organized microcrystalline web-shaped structure with a degree of polymerization of 8000 providing porous geometry, crystallinity, water holding capacity and high mechanical strength (Laavanya et al., 2021). BC, due to its nature, finds application in a wide range of biomedical applications, packaging and pharmaceutical wound healing agents (Keshk, 2014; Cacicedo et al., 2016). Presently, lab or industrial scale facilities producing BC mostly utilizes high glucose/fructose based synthetic culture medium, which makes the entire process costly and economically infeasible (UI-Islam et al., 2017). One alternative to reduce the cost of production is to use cheaper resources as substrates like waste streams of agro-industries, breweries, bakery and textile mills and municipal waste for production or apply the circular biorefinery concept to harness multi bio-based products (Ul-Islam et al., 2020).

Kombucha tea is a non-alcoholic beverage obtained from the fermentation of a sweetened tea mixture inoculated with mixed cultures of acetic acid bacteria and osmophilic yeast for approximately a period of 10–14 days (Emiljanowicz and Malinowska-Pańczyk, 2020). The microbiome present in the beverage contributes to its probiotic property and in recent times it is globally being considered under the category of functional beverages (Sinir et al., 2019). The Kombucha beverage industries have started booming and have already occupied substantial market value and is predicted to increase further in future (Ramírez Tapias et al., 2020). A litre of Kombucha beverage is being sold at an average rate of 7 US \$ to 9 US \$. In a cost and energy analysis study done for Kombucha production process, it was reported that the total production cost was 0.97 US \$ per litre with a gross profit value of 1.35 US \$ per litre and a benefit to cost ratio of 1.39 (Mohammadshirazi and Kalhor, 2016).

During the fermentation process, there is a continuous production of extracellular cellulose biofilm layers by the acetic acid bacteria present in the culture at the air-liquid interface (Amarasekara et al., 2020). The yeast cells of the inoculum metabolize the sugar and bacterial cells, consume it for the primary and secondary metabolic pathways, producing a gelatinous biofilm or a zoogleal mat also called Symbiotic Culture of Bacteria and Yeast (SCOBY) that floats at the air-liquid interface for sufficient oxygen supply (Laavanya et al., 2021). Often the Kombucha based bacterial cellulose (KBC) biofilms have similar characteristic mechanical, physical, chemical and biological properties like tensile strength, adsorption capacity, high melting temperature, biodegradability, etc.as that of the BC and could be used in environmental biotechnology (Najafpour et al., 2020), textiles (Kamiński et al., 2020) and biomaterial industries (El-Wakil et al., 2019). Unaware of the benefits, very-often the Kombucha beverage industries have been discarding the SCOBY as a waste or partly utilizing it as an inoculum for the fresh batch of fermentation. Alternatively, the KBC can be used as a cheaper source of cellulose for industrial purpose.

Several researchers have analyzed the influence of operational factors on KBC yield (Treviño-Garza et al., 2020; Villarreal-Soto et al., 2021) and have also reported the physical and mechanical characteristics (Avcioglu et al., 2021; Bertsch et al., 2021) of these biofilms. Large scale production of Kombucha beverage is seen as a commercially feasible and profitable business whereas production of the by-product i. e., the Kombucha cellulose biofilm as a main stream product for utilization in various applications has to be yet studied thoroughly. Technoeconomic analysis of a process is generally done to assess the feasibility of technical data such as lab-scale cultivation and yield data with the economic value of the same process during the large-scale production (Kim and Adhikari, 2020). The techno-economic feasibility studies related to the Kombucha beverage fermentation or bacterial nanocellulose are extremely limited. Mohammadshirazi and Kalhor, (2016) analyzed the process related to Kombucha beverage fermentation and reported that the input costs can be reduced by increasing the volume of production. Dourado et al. (2018) through economic feasibility and

process modelling of bacterial nano-cellulose fermentation process reported low yield and high capital investments and operating costs are the major constraints to the commercialization of bacterial nano-cellulose. Even-though the cultivation methods, reactor designs, growth and maintenance of KBC is being studied well, the large-scale production is often hindered due to the lack of information related to the process economic feasibility. The technicalities like yield, productivity along with economic feasibility and profitability of cultivation should be considered during scale-up and commercialization.

To the best of authors knowledge, till now there has not been any studies regarding large scale production and techno-economic feasibility analysis of the Kombucha cellulose biofilm production. Thus, the aim of the present study is to simulate a process model and study the techno-economic feasibility of large-scale Kombucha cellulose production process. The input data for the simulation has been collected by lab experimentation under optimized process conditions with several replicates. The economic process feasibility was evaluated in terms of the net present value (NPV) and the payback time of the project. Such studies will help in realizing the overall economic feasibility of the project before implementation at real time scale.

2. Methodology

2.1. Process description

The Kombucha cellulose production consists of the fermentation process with the utilization of sugar (commercial edible sugar) as the carbon source, black/green tea leaves as the source of polyphenols and other metabolites/protein to form the cellular machinery along with the previously fermented Kombucha broth and symbiotic culture of bacteria and yeast acting as the starter culture or the inoculum. The cellulose sheet obtained is often bleached with the application of sodium hydroxide to remove the residual sugars, bacterial and yeast cells, other contaminants, melanoidins, followed by repeated washing with water to remove the trace alkali deposition (Laavanya et al., 2021). The present study is based on a step-by-step production and recovery of Kombucha cellulose sheets involving 5-unit process spanning from the upstream to downstream technologies. The entire bioprocess consisted of the upstream process of i). Seed fermentation and inoculum preparation followed by ii). The static fermentation and then, the downstream processing involving iii). Washing with sodium hydroxide iv). Drying in a tray dryer and the final step involving v). Extrusion into thin biofilm based filaments.

The inoculum was prepared in a seed fermenter with commercially available sugar and waste tea leaves solution (prepared by boiling the tea processing industry waste) as the growth medium along with the previously fermented Kombucha tea (starter inoculum). Seed fermenter was operated for a period of 14 days at 30 °C. The inoculum from the seed fermenter was fed into the static fermentation unit, and was mixed with more substrate (sugar and tea solution) facilitating the formation of Kombucha based cellulose biofilm. The static fermentation was also assumed to be done for a period of 14 days at temperature of 30 $^{\circ}$ C. The liquid broth containing sugars as well as prebiotic bacteria and osmophilic yeast was separated in the top stream of the static fermentation unit and can be marketed as the Kombucha tea (fermented beverage) to be used as a health drink for stimulating the digestive metabolism and boosting the overall immunity. The cellulose biofilm formed was passed onto the belt filter and bleached washed with 0.5 M sodium hydroxide solution for removal of residual sugars, microbial cells and other contaminants. The cellulose biofilm was then dried in a tray dryer at 60 $^{\circ}\text{C}$ and extruded into filament form which can be marketed directly. The representation of the process flow with system boundary is shown in Fig. 1.

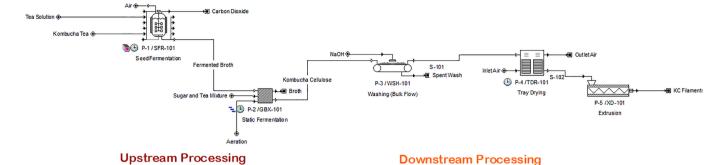


Fig. 1. Schematic process flow diagram for the Kombucha cellulose based biofilm production and its processing into filaments.

2.2. Techno-economic performance evaluation

2.2.1. Process simulation description

The process pathway involving the Kombucha cellulose biofilm formation during fermentation followed by the washing, and drying was simulated as a 5-unit step process to study the economics involved using *SuperPro* Designer v10 (Intelligen Inc.) with the base prices as of year 2021. The schematic process flow methodology is shown in Fig. 1.

The upstream process involved inoculum preparation in seed fermenter units with a maximum volume of 250 L and the working volume of 182 L. Following this, to meet the production demand as per the flow rate, Kombucha fermentation was carried at 30 $^{\circ}$ C in 10 extra static reactors under staggered mode each with a rated throughput of 1546 kg/h, for promoting biofilm propagation. Equipment occupancy chart showed that the fermentation step is the major scheduling bottleneck, occupying the maximum time per batch compared to other equipment in the process. To perform adequate adjustment for obtaining higher yield and throughput, the particular process was simulated in staggered mode. The system is assumed to be operated round the clock in 24 h cycle, with one fermentation batch initiated and another completed daily with a total processing time of 14 days. Similar reactors operated in clean room under staggered mode was also reported by Dourado et al. (2018) for bacterial nano-cellulose production. The output from the static fermenter is then directed into the downstream processing units which operates continuously. The fermentation broth from the static fermentation unit can be marketed as Kombucha tea serving as the secondary source of revenue for this study.

The upstream process in the seed fermenter and the static fermentation unit involves a series of reactions based on the inherent metabolism of the SCOBY to convert, the sugar (sucrose) into cellulose and other by-products as outlined from equation Eq. (1) to Eq. (4).

$$C_6H_{12}O_6(Sucrose) + H_2O(Water) \rightarrow C_6H_{12}O_6(Glucose) + C_6H_{12}O_6(Fructose)$$
 (1)

$$C_6H_{12}O_6(Glucose) + C_6H_{12}O_6(Fructose) \rightarrow (C_6H_{10}O_5)_n(Cellulose)$$
 (2)

$$C_6H_{12}O_6(Glucose) \rightarrow CO_2(Carbondioxide) + C_2H_5O_{10}(Ethanol)$$
 (3)

$$C_2H_5O_{10}(Ethanol) + O_2(Oxygen) \rightarrow CH_3COOH(Aceticacid) + H_2O(Water)$$

Kombucha based bacterial cellulose biofilms from here are assumed to be directed onto a belt filtration unit, with a volumetric flow rate of 1 L/h and a rated throughput of 7.58 kg/h to facilitate bleaching with sodium hydroxide treatment. The bleached biofilm obtained were then dried at 60 $^{\circ}\text{C}$ in the tray dryer (1140 L volume) having trays with an area of 5 m² and tray to tray vertical distance of 25 cm each. The dried Kombucha cellulose based biofilm obtained was passed onto the extrusion device with a screw diameter of 0.91 cm and metering depth of 6 mm to form filaments.

The input and the flow rate for the seed fermenter as well as the static

fermentation unit was based on the laboratory scale experimental data (one variable at a time pre-optimization) showing the maximum yield of 779 g wet Kombucha cellulose/3 L was obtained with 80 g/L sucrose, 12 g/L tea with 10% v/v of Kombucha tea.

Surface morphology of KBC biofilms (5 mm length * 5 mm breadth) glued on carbon tape coated with platinum was determined by using the scanning electron microscope equipped with an energy dispersive X-ray (SEM-EDX) facility at a voltage of 15 kV in a 2° electron contrast mode at 2500X magnification.

2.2.2. Economic evaluation

The input data including the raw material costs, unit operations and reaction kinetics are used as the baseline specified by the SuperPro designer software to simulate the number of equipment desired. Table 1 shows the units of each equipment, material used, the size and the capital cost during each process for the design simulation. The total capital costs are the sum of the direct fixed capital, the working capital as well as the start-up cost involved. Direct fixed capital includes the equipment price, along with the installation, piping, electricity, yards improvements and other auxiliary costs. The calculation factors or multiplier value were set at the default level as stated by Peters et al. (2003). The total operating costs are the sum of the raw material costs, the labor charges, facility dependent and utility costs including the price of electricity consumed. Raw materials included are sugars and tea mixture (0.6 US \$ per kg) and sodium hydroxide (0.38 US \$ per kg). The revenues consisted of two streams i.e. 1). Kombucha cellulose based filaments (as the main revenue stream) and 2). Kombucha broth/ beverage as the secondary stream. The Kombucha beverage was assumed to be sold at 8.70 US \$/kg (Kim & Adhikari, 2020). The Kombucha cellulose based filaments was assumed to be sold at the price of 1.63 US \$/kg. The profitability analysis of the process simulation was done with the minimum selling price, unit production costs, gross profit, net profit, Net Present Value (NPV), Internal Rate of Return (IRR), Payback period and Return on Investment (ROI) via the cash flow analysis. The profitability parameters were calculated considering an operational time of 330 days, 40% taxes, with an inflation of 4% and at an interest rate of 7%.

2.3. Assumptions and limitations of the model

The entire facility was assumed to be run for 330 days per year with the remaining days of a year left for the maintenance of the plant. The entire bioprocess has been assumed to follow the hazard analysis critical control points (HACCP) based approach during Kombucha fermentation as outlined by Murphy et al., (2018). With a batch hold-up period of 14 days and hence approximately 23 batches per year can be run. The net profit of the process was calculated considering 40% taxes, after subtracting the operating costs from the total revenues. Cash flow analysis was done following straight line depreciation method over a period of 15 years, with the initial 3 years as the start-up period for the company.

Table 1Equipment description, units and price contributing to the capital expenditure.

Name	Description	Material	Units	Unit Cost (\$)	Cost (\$)
SFR-101 Seed Fermenter		SS 316	18	22,000	396,000
	Vessel Volume $= 242.71 L$				
GBX-101	Static Fermenter	CS	10 staggered units	1,05,000	1,155,000
	Rated Throughput = 1546.21 kg/h				
WSH-101	Washer (Bulk Flow)	CS	1	20,000	20,000
	Rated Throughput = 7.58 kg/h				
TDR-101	Tray Dryer	SS 316	1	68,000	68,000
	Tray Area $= 4.56 \text{ m}^2$				
XD −101	Extruder	SS 316	1	58,000	58,000
	Screw Diameter $= 0.91$ cm				
	Unlisted Equipment				413,000
				TOTAL	2,110,000

^{*}Abbreviations: SS: Stainless steel; CS: Carbon steel.

3. Results and discussion

3.1. Yield and properties of Kombucha cellulose biofilm

Kombucha cellulose based bacterial biofilm is usually produced during Kombucha tea fermentation, where the black tea and sucrose are used as the traditional substrate. To reduce the cost of production, waste tea leaves from tea processing industries were used in the study to provide the polyphenol and nitrogenous compounds necessary for KBC biofilm growth. The optimization experiments in the present study at the lab-scale level were done with a working volume of 3 L. The total weight of wet Kombucha cellulose obtained was 779 g (yield of 3.246 g/g and a maximum volumetric productivity of 18.54 g/L day) with thickness $7.87 \, \text{mm}$ at the optimal media composition of $1.2\% \, \text{w/v}$ tea leaves, $80 \, \text{g/}$ L sucrose (commercial edible sugar), 10% v/v Kombucha tea and 5% w/vv SCOBY as inoculum after a period of 14 days. Similar yield of KBC was also reported by Al-Kalifawai and Hassan (2014). Often an appropriate concentration of substrate is essential to facilitate bacterial cellulose formation. Surface morphology analysis of KBC biofilms performed through the SEM showed the presence of cellulosic fibrils surrounded by the homogenous distribution of melanoidins that strongly intercalate these fibres together forming an intricate cellulose network. These are usually referred to as bacterial nanocellulose fibers and attributed to have high surface area with fiber diameter ranging from 20 to 100 nm (Sharma and Bhardwaj, 2019). Pogorelova et al. (2020) reported bacterial cellulose microfibers in bundles with intracellular space/gaps in between through SEM analysis. Kumar et al. (2019) have also reported the presence of fine cellulosic fibrils arranged irregularly in bacterial cellulose biofilm via scanning electron microscopic analysis. The EDX analysis showed the significant presence of carbon and oxygen, which is the characteristic feature of cellulosic biofilm. Similar to the present study, 44% carbon along with 49% oxygen has also been observed during the EDX analysis of bacterial cellulose by Pogorelova et al. (2020).

3.2. Assessment of the capital expenditures

The total direct fixed capital expenses (Table 2) comprise of the total plant direct capital costs; and indirect capital costs linked with the construction and engineering of the plant and the contractor's and the contingency fees. Direct fixed capital costs of the process are mostly associated with the equipment prices, apart from the indirect costs linked with the installation, piping and other auxiliary activities. Here, the total equipment purchases costs amounted to 2.1 million US \$. The equipment for downstream processing i.e. the belt filter (bulk washing); the tray dryer and the extrusion device and occupy a maximum share (69%) of the total expenses, followed by the static fermentation unit which accounts for 54.7% of the total capital costs. The summary of the cost structure involved showed that the direct costs involving the equipment costs, the installation, process piping, instrumentation,

 Table 2

 Capital expenditure of the Kombucha cellulose biofilm production process.

Fixed capital estimate summary (2021 prices in \$)						
A. Total Plant Direct Cost (TPDC)						
1. Equipment Purchase Cost	2,110,000					
2. Installation	967,000					
3. Process Piping	739,000					
4. Instrumentation	844,000					
5. Insulation	63,000					
6. Electrical	211,000					
7. Buildings	950,000					
8. Yard Improvement	317,000					
9. Auxiliary Facilities	844,000					
TPDC	7,045,000					
B. Total Plant Indirect Cost (TPIC)						
10. Engineering	1,761,000					
11. Construction	2,465,000					
TPIC	4,226,000					
C. Total Plant Cost (TPC = TPDC + TPIC)						
TPC	11,271,000					
D. Contractor's Fee & Contingency (CFC)						
12. Contractor's Fee	564,000					
13. Contingency	1,127,000					
CFC	1,691,000					
E. Direct Fixed Capital Cost (DFC = TPC + CFC)						
DFC	12,962,000					

^{*}The multiplier and the cost factor for each of the cost parameter has been referred from Peters et al., (2003).

insulation, electrical, building and yard improvements occupy the majority of share for expenses for the total plant costs. Similar conclusions can also be drawn from the study by Dourado et al. (2018) for bacterial nano-cellulose fermentation. The authors reported a total direct capital cost for the project to be 1.3 million US \$, with 70% of the costs linked to installation, process piping, instrumentation, insulation, electrical building, yard improvements and other auxiliary facilities. Similar to the above study, Ul-Islam et al. (2020) reported that 71% of capital expenses for bacterial cellulose production were linked to the total plant direct costs.

The total plant costs (11.27 million US \$) included the total plant direct costs and indirect costs i.e., the indirect costs involved in construction and engineering (4.22 million US \$ i.e. 37.5% of total plant costs). The direct fixed capital costs also comprise of the contractor and contingency fees with the total plant costs, thus totaling to 12.96 million US \$. The contingency charges added into the project estimates aids in accounting for any unpredictable variations in the costs due to minor fluctuations in price and the process. While the present study reported 33% of direct capital costs associated with the plant indirect capital expenditure, Ul-Islam et al. (2020) projected 29% share of capital costs linked to engineering and construction. The study by Dourado et al. (2018) also reported similar estimates for bacterial nano-cellulose production including the steps of size reduction and product packaging.

Vanhatalo et al. (2014) reported 3.4 million Euros/year capital costs and an incurred fixed costs of 4 million Euros/ year for microcrystalline cellulose production process. The variation in the project costs are mostly related to the differences in the system boundary and the unit process considered during the study.

3.3. Assessment of the operational expenditures

Along with the capital expenses, operating costs also play a prominent role in determining the profitability of biotechnologically driven process like here the Kombucha based cellulose biofilm formation. A total operating expenditure of 3.83 million US \$ was estimated for the present study. Dourado et al. (2018) reported 5.3 million US \$ during the manufacturing of bacterial nano-cellulose via fermentation. On the other hand, Ul-Islam et al. (2020) reported 7.4 Million US \$ to be associated with the manufacturing of bacterial cellulose. The lower operational/manufacturing costs obtained in the present study compared to the previous studies might be attributed to the utilization of waste resources and cheaper raw materials.

The breakdown of the operating expenses incurred during the project included the raw materials, consumables, labor, facility and utility dependent charges. Maximum expenditure of 2.45 million US \$ is associated with the facility dependent costs linked with the bioprocessing steps including the fermentation facilities, washing, drying and extrusion units. The labor costs occupy the next majority of the operating share (23%) due to the prices of operator and supervisor costs associated during the batch processes. The utility costs, mainly the electrical energy accounts for 6% of the total operational costs. Raw materials occupy 2% of the total operating costs with maximum share by sucrose (77%), Kombucha tea broth/inoculum (10%), sodium hydroxide (8.57%) and tea (3.05%). Consumables like the belt filter membrane and lubricants utilized during dewatering, along with the shake flasks occupies 2% of the total share of expenditures. The recent study by Ul-Islam et al. (2020) also projected labor charges occupy a significant portion of the total direct costs during the bacterial cellulose production process. Mohammadshirazi and Kalhor, (2016) reported that highest share of 23% was associated with the machinery or facilities) followed by human labor that occupied 18% of the total operational cost expenditure during the techno-economic assessment of Kombucha beverage production. The study also reported the highest raw material costs to be linked with the sucrose (sugars) followed by the Kombucha tea broth during fermentation as also projected in the present study.

3.4. Profitability assessment of the project

Profitability analysis of the project is done in order to estimate whether the bioprocess scenario simulated will be feasible during the real-time implementation. In the present study, profitability of the project, as represented in Table 3, was assessed by considering the filaments formed after extrusion of the KBC biofilm as the primary source of revenue and the fermented tea (Kombucha broth) as the secondary source of revenue. The total production capacity of the process is 60023 kg (extruded filaments)/year or 60.023 tons (extruded filaments)/year. The total investment involved was predicted to be 13.72 million US \$ with a unit production cost of 63.83 \$/kg and an annual operating cost of 3.8 million US \$. Since, the unit production revenue amounts to 119.67 \$/kg which is 87% more than the unit production costs involved, the overall process is profitable. With a gross profit margin of 46.66%, the invested project could possibly result in 23.64% return on investment. The internal rate of return after taxes was 16.48% and the net present value at 7% interest rate amounted to 9 million US \$. The cash flow analysis shown in Table 4 also projected positive cash flow and profits with a payback period of 4.23 years, after which the company would be able to recover the spent amount. Mohammadshirazi and Kalhor, (2016) reported a gross return of 0.77 \$/L and a net return of 0.38 \$/L for Kombucha beverage production. Net profits totaling to 3.3

Table 3Profitability analysis of Kombucha cellulose biofilm production process.

Sl. No.	Categories	Amount
1.	Direct Fixed Capital (A)	12,962,000 \$
2.	Working Capital (B)	109,000 \$
3.	Start-up Cost (C)	648,000 \$
4.	Total Investment $(A + B + C)$	13,719,000 \$
5.	Annual Operating Costs (D)	3,831,000 \$
6.	Annual Revenue from KBC biofilm (E)	98,000 \$
7.	Annual Revenue from Kombucha Beverage (F)	7,085,177 \$
8.	Total Revenues Earned per Year $(G = E + F)$	7,183,177 \$
9.	Net Unit Production Cost (per kg)	63.83 \$
10.	Net Unit Production Revenue (per kg)	119.67 \$
11.	Gross Profit per Year $(H = G - D)$	3,352,177 \$
12.	Taxes (in 40%) per year (I)	1,341,000 \$
13.	Net Profit (H $-$ I $+$ Depreciation)	3,242,000 \$
14.	Gross Margin	46.66%
15.	Return on Investment	23.64%
16.	Payback Time	4.23 Years
17.	Internal Rate of Returns (IRR) after Taxes	16.48%
18.	Net Present Value (NPV) at 7% Interest	9,087,000 \$

million US \$ and return on investment of 10% for the production and sale of bacterial nano-cellulose cube slices were projected by Dourado et al. (2018). Ul-Islam et al. (2020) also reported a profit 3.3 million US \$, with a payback period of 4 years to recover the amount invested during the project tenure.

3.5. Sensitivity analysis of the Kombucha biofilm production process

The Kombucha based cellulose production is dependent over the process conditions, which in-turn influences the unit production costs and the economic feasibility. Sensitivity analysis is often employed to analyze the variation in prices with changes in operational conditions and revenues over the process economics. The sensitivity analysis to access the economic feasibility under varying operational conditions as represented in Fig. 2. It was observed that increasing volume of seed fermentation unit can decrease the number of equipment needed during the inoculum propagation stage and bring down the unit production costs, ensuring higher net present value and low payback time. Maintaining a seed fermenter volume of 250 L provided a unit production cost of 63.83 \$/kg as the base case and would result in a NPV of 9.08 million US \$ having a payback time of 4.23 years. Decreasing the seed fermenter volume to 100 L will decline the NPV to 4.2 million US \$, thereby increasing the payback time to 5.71 years. On the other hand, increasing the seed fermentation capacity to 1000 L will enhance the NPV to 11.51 million US \$ decreasing the payback time to 3.52 years. Thus, the economics of the process was found to be dependent on the facility and the unit operations involved. Mohammadshirazi and Kalhor, (2016) also reported that the input costs of Kombucha fermentation can be reduced by increasing the volume of production. Similarly, it was shown that increasing the raw material costs by 25%, will decrease the NPV to 9.00 million US \$ and with 64.11 \$/kg of unit production cost, payback period is expected to slightly increase to 4.24 years. Gao et al. (2021) reported that the utilization of alternative cheaper media components can reduce the cost of bacterial cellulose fermentation. Ye et al. (2019) also reported the utilization of cheaper carbon substrate for favoring the bacterial cellulose production. On the other hand, decreasing the sugar source cost by 25%, with the use of recycled stream of Kombucha tea as starter culture and water for washing is expected to increase the NPV to 9.20 million US \$ (63.39 \$/kg unit production cost) decreasing the payback time to 4.21 years. Further, increasing the labor cost by 25% will result in an increment in the unit production cost and payback time to 78.96 US \$/kg and 5.11 years respectively along with the decline in NPV to 4.5 million US \$, while a decline in unit production cost and payback time to 46.88 US \$/kg and 3.54 years respectively with NPV of $14.15\,million$ US \$ was observed with 25% decrease in labor costs. Thus, automation of the reactor can also be proposed as a strategy to reduce

Table 4
Cash flow analysis (in thousand US \$) for Kombucha cellulose biofilm production process.

Year	Capital Investment	Debt Finance	Sales Revenues	Operating Cost	Gross Profit	Loan Payments	Depreciation	Taxable Income	Taxes	Net Profit	Net Cash Flow
1	- 3,888	0	0	0	0	0	0	0	0	0	- 3,888
2	- 5,185	0	0	0	0	0	0	0	0	0	- 5,185
3	- 3,998	0	1,197	3,414	- 2,217	0	1,231	0	0	- 986	- 4,983
4	0	0	7,183	3,831	3,352	0	1,231	3,352	1,341	3,242	3,242
5	0	0	7,183	3,831	3,352	0	1,231	3,352	1,341	3,242	3,242
6	0	0	7,183	3,831	3,352	0	1,231	3,352	1,341	3,242	3,242
7	0	0	7,183	3,831	3,352	0	1,231	3,352	1,341	3,242	3,242
8	0	0	7,183	3,831	3,352	0	1,231	3,352	1,341	3,242	3,242
9	0	0	7,183	3,831	3,352	0	1,231	3,352	1,341	3,242	3,242
10	0	0	7,183	3,831	3,352	0	1,231	3,352	1,341	3,242	3,242
11	0	0	7,183	3,831	3,352	0	1,231	3,352	1,341	3,242	3,242
12	0	0	7,183	3,831	3,352	0	1,231	3,352	1,341	3,242	3,242
13	0	0	7,183	2,600	4,583	0	0	4,583	1,833	2,750	2,750
14	0	0	7,183	2,600	4,583	0	0	4,583	1,833	2,750	2,750
15	757	0	7,183	2,600	4,583	0	0	4,583	1,833	2,750	3,507

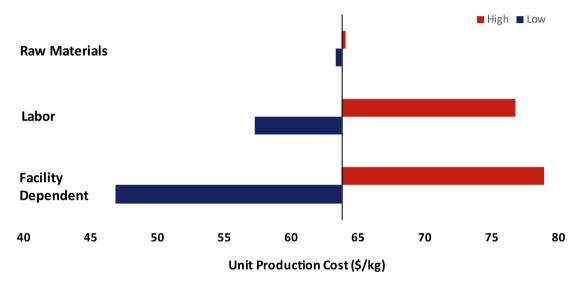


Fig. 2. Sensitivity analysis of the Kombucha cellulose based biofilm production and its processing into filaments.

the manual labor charges, making the process more economically sustainable. Technological demand, process simplification and automation is expected to improve the usage strength of labor and thereby enhance their socio-economic status.

Multiproduct biorefinery is often used to harness economic advantages out of biotechnological process by selling several products formed in different unit process of an entire process chain. It was evident that while considering the KBC filaments and Kombucha beverage as the source of revenue, a unit production revenue of 119 \$/kg can be obtained per year, and the company can start earning profits after 4.23 years. The unit production revenue was drastically reduced to 1.63 \$/kg, instead of 119 \$/kg while considering the Kombucha cellulose based filaments as the only revenue source, making the NPV of the process negative, hence economically unfeasible. Filippi et al. (2022) also proposed that extraction and exploration of several bio-based products in a biorefinery including the production of bacterial cellulose from grape pomace can make the process cost-competitive. Techno-economic studies performed by Ratshoshi et al. (2021) for production of two high value-added products biopolymers by utilizing waste industrial stream as raw material similar to the current study explained the important role played in building energy self-sufficient biorefinery due to low process energy demands. Shahzad et al. (2017) explained the importance and techno-economics of utilizing diverse industrial waste as raw materials for production of polyhydroxyalkanoate (PHA). The study elaborated on the need for cost-effective alternatives for nutritionally important carbon sources like carbohydrates and edible vegetable oils which are currently being used in PHA production. The above-mentioned study projected an overview on varying price of PHA based on fluctuating market prices of the input raw material streams elaborated on the impact of the annual revenue generation and investment payback period in near future. Since Kombucha-based cellulose production also depends on edible carbohydrate raw material, different alternative cheap resources can be tested for decreasing process costs in input side and parallelly reusing waste industrial streams to produce value-added products. Similar conclusions have also been reported in the study by Ul-Islam et al. (2020) where the low cost substrates from agro-industrial wastes could be utilized to produce good quality bacterial cellulose at comparatively lower price than synthetic chemicals.

4. Conclusion

The present study analyses the techno-economic feasibility of KBC biofilm production and conversion into filaments. Unit production cost of 63.83 US \$/kg was estimated with facility dependent and labor charges occupying a major share of KBC biofilm based extruded filament production. With annual unit production revenue of 119.67 US \$/kg, the process could be profitable with NPV of 9.08 US \$ and payback time of 4.23 years. The sensitivity analysis revealed that increasing the fermentation volume with decline in labor via process automation and with the sale of multiple products can improve the economics, making it

suitable for future commercialization.

CRediT authorship contribution statement

Bunushree Behera: Conceptualization, Writing – original draft. **D.** Laavanya: Data analysis, Writing – original draft. Paramasivan Balasubramanian: Conceptualization, Investigation, Funding acquisition, Writing – review & editing.

Declaration of Competing Interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2021.126659.

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