Production of sphere-like bacterial cellulose in cultivation media with different carbon sources: a promising sustained release system of rifampicin

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Abstract

Bacterial cellulose (BC) production can be performed using a static or agitated culture method. In the static culture method, the BC is obtained presents three-dimensional thinner network structures and excellent mechanical properties. In the agitated culture method, BC is produced in the form of granules or fibrous threads with a lower degree of polymerization, mechanical strength, and crystallinity than those formed in static fermentation. Compared with BC membranes, sphere-like BC (SBC) cultured under agitated conditions showed advantages for adsorption due to its larger surface area. The objectives of this work were to obtain SBC, by the bacterial strain *Komagataeibacter hansenii* ATCC 23769, in agitated cultivation, using media containing different carbon sources carbon sources, such as fructose (FRU), glucose and sucrose (MS1), sucrose (Y) and glucose (Z and HS), aiming to produce supports for sustained release of rifampicin (RIF). SBC has been produced under agitation at 130 rpm and 25°C. SBC obtained were processed to remove bacteria and residues from the culture media and lyophilized. The SBC characterizations were performed by Fourier transform infrared spectroscopy, X-ray diffraction, Field emission gun-scanning electron microscopy, thermogravimetric, and by derivative thermogravimetry analysis. The SBC produced were impregnated with antibiotic RIF and tested for the sustained release capacity of this drug by diffusion method and Frans cell kinetics. SBC that the best results for all tests were produced in FRU, Z and MS1 media, respectively. The results demonstrate the potential of the SBC to contribute to the design of new drug delivery

systems with biomedical applications.

1. Introduction

Cellulose is a biopolymer that can be produced by several species such as plants, algae and some bacterial genera such as *Rococo's* (Tanskul et al., 2013), *Acetobacter*, *Rhizobium, Agrobacterium*, *Aerobacter*, *Achromobacter*, *Azotobacter*, *Salmonella*, *Escherichia* and *Sarcina* (Islam et al., 2017; Ullah et al., 2016).

First described in 1886 by Brown, bacterial cellulose (BC) proved to be a biopolymer of great interest for application in several industrial and medical areas, due to its structural characteristics, which prove to be advantageous in relation to plant cellulose (PC) (Cacicedo et al., 2016; Shah et al., 2013; Ul-Islam et al., 2012a). Although its structure is identical to that of PC, consisting of β-(1→4) glycosidic bonds, BC is devoid of lignin, pectin and hemicellulose, which gives it a high degree of purity(Kumar et al., 2019). A special emphasis has been given to the cellulose produced by bacteria of the Acetobacteraceae family mainly of the *Gluconacetobacter* (Cacicedo et al., 2016) genus, later named *G. xylinus* and *G. hansenii*, and currently classified in the genus *Komagataeibacter* (Yamada, 2014), using a variety of natural and synthetic culture media, with different carbon sources (Iguchi et al., 2000; Lazarini et al., 2018, 2016; Lustri et al., 2015; Shah et al., 2013; Ul-Islam et al., 2012b, 2012a)

Hydrated BC membranes have a high capacity for adsorption of different ionic and molecular species or even particle stabilization, as they have a highly hydrated structure of nanometric fibers forming a highly porous system (Eichhorn et al., 2010; Iguchi et al., 2000). Due to high mechanical resistance, hypoallergenicity, biocompatibility and high degree of liquid absorption(Lazarini et al., 2018, 2016), the BC are widely studied as controlled drug delivery systems, by our research group (de Oliveira Barud et al., 2016; Lazarini et al., 2018, 2016; Machado et al., 2018) and many international groups (Badshah et al., 2018; Fontes et al., 2017; Juncu et al., 2016; Mohd Amin et al., 2014; Negut et al., 2018; Treesuppharat et al., 2017; Ullah et al., 2016; Urbina et al., 2020).

The BC production can be performed using a static, agitated, or using a bioreactor fermentation method. In the static culture method, the BC is obtained in as a film at the air-liquid interface, promoting thinner three-dimensional network structures and excellent mechanical properties(Wang et al., 2019). In the agitated culture method, BC is produced in the form of fibrous granules or threads with a lower degree of polymerization, mechanical resistance, and crystallinity than the films formed in static fermentation. However, agitated culture is widely used for commercial purposes because it produces BC in less time, as well as possesses economic viability and desired applications (Shah et al., 2013), such as drug release (Wang et al., 2018). Bioreactor using method, which can employ both static and agitated cultures, being an alternative method classified in terms of the use of oxygen-enriched air, a rotating disc, or biofilm support, equipped with a rotating filter or a silicone membrane (Islam et al., 2017; Wang et al., 2018).

Czaja et al. (Czaja et al., 2004) proposed that the continuous shear force during agitation caused the cellulose ribbons to intertwine with each other to form the sphere-like structure.

BC membrane has widely acknowledged as biopolymer with large surface area and 3D nano-pores structure that promotes high capacity for absorbing liquids (Lin et al., 2013; Meng et al., 2019b). Compared with BC membranes, sphere-like BC (SBC) cultured under agitated conditions showed great advantages for adsorption due to its larger surface area (Diaz-Ramirez et al., 2021; Iguchi et al., 2000; Meng et al., 2019b).

Hoshi et al. (Hoshi et al., 2018) and Wang et al. (Wang et al., 2019) reported that the formation of SBC depends on the bacterial strain used, that is, not all species are capable of producing spheres in an agitated medium and the agitation speed promotes great interference in the formation of fiber networks. Thus, depending on the speed, obtaining more homogeneous spheres can be easily acquired, presenting perfect applicability for drug release.

Study, performed by Meng et al. (Meng et al., 2019a) using various bacterial culturing parameters were assessed with the goal of assembling uniform SBC which has advantages owing to its unique morphology and increased surface areas. The results showed that the uniform SBC was synthesized when the ratio of culture medium to flask volumes (M/F value) was kept at 50%, while the diameter of these uniform SBC could be adjusted by changing the agitation speed and flask volume.

Therefore, SBC could hold huge potential for many high value applications such as slow drug release (Abeer et al., 2014; Meng et al., 2019a). However, the control of properties shape, diameter, moisture content, adsorption ability, surface area and its uniformity of the SBC are a challenge to be overcome (Meng et al., 2019b).

In addition to different BC production methods, it is interesting to evaluate the effects of using different carbon sources for BC production (Fernandes et al., 2020). Several culture media are reported in the literature for the production of BC as Hestrin-Schramm (HS) (Hestrin and Schramm, 1954), Zhou (Z) (Zhou et al., 2007) and Yamanaka (Y) (Yamanaka et al., 1989). The BC production in this different culture media showed different dry mass yield and physical-chemical characteristics (Mohammadkazemi et al., 2015).

RIF, a semisynthetic antibiotic obtained from rifamycin (Sutradhar and Zaman 2021) which has bactericidal activity by inhibiting bacterial RNA polymerase and with capacity to penetrate through the biofilm (Alifano et al., 2014; Politano et al., 2013), is used as a first line treatment for tuberculosis, as well as in the treatment of other infectious diseases (Sutradhar and Zaman, 2021) and constitute one of the most potent and broad-spectrum antibiotics against others bacterial infection, as endocarditis caused by Gram-positive methicillin-susceptible or resistant *Staphylococcus* spp, pneumonia, particularly ventilator-associated, caused by *S. aureus* and Gram-negative bacilli such as *Pseudomonas aeruginosa* and Enterobacteriaceae and *Acinetobacter* spp. (Lee et al., 2017).

Conventional dosage forms for immediate local drug release, such as solutions, creams, ointments, gels and adhesives do not significantly interfere with the release of therapeutic agents. However, controlled and sustained release systems are products with advanced therapeutic performance, modulating the release and controlling the time and place in which the drugs are released (Carvalho et al., 2021). Thus, reservoir or matrix systems, where drugs are dispersed, contained in a nucleus, or fixed to the surface, have aroused interest (Carvalho et al., 2021, 2020) .

The present work had as aims the production and characterization of SBC, by *K. hansenii* ATCC 23769, using culture media with different compositions and concentrations of carbon and nitrogen sources (FRU, and MS1) compared to culture media already used in BC production (HS, Y, and Z) to study the influence on the physicochemical properties of the SBC produced and its possible relationship with the ability to retention and sustained release of the antibiotic RIF, a broad spectrum antibiotic, to future use in medicine. The obtained SBC were characterized by Field emission gun-scanning electron microscopy (FEG-SEM), Fourier transform spectroscopy (FTIR), X-ray diffraction (XRD) and Thermogravimetric and derived thermogravimetry (TGA/DTG) analysis, and the RIF sustained release capacity was evaluated by method and diffusion release kinetics by Franz cells.

2. Materials And Methods

2.1. Materials

The bacteria for SBC production *Komagataeibacter hansenii* ATCC 23769 and *Staphylococcus aureus* ATCC 25923 used for rifampicin sustained release test were purchased from André Tosello Foundation. Anhydrous glucose, fructose, sucrose, and ethanol were purchased from Synth, yeast extract, peptone, bacteriological agar, and Müller-Hinton agar were purchased from Kasvi. Corn Steep Liquor and rifampicin were purchased from Sigma-Aldrich. Citric Acid, Na_2HPO_4, KH_2PO_4, $(NH_4)SO_4$, and $MgSO_4$ $.7H_2O$ were purchased from Merck.

2.2. Methods

2.2.1. SBC production and processing

K. hansenii ATCC 23769 was reactivated from stock in glycerol stored at -80ºC, in a medium FRU (Table 1) being kept in a Biological Oxygen Demand (B.O.D.) oven at 28ºC until the growth of a BC membrane, for 7 days. Afterwards, the culture was vigorously shaken to obtain bacterial suspension the was used as pre-inoculum to produce SBC. From the pre-inoculum, was produced a bacterial suspension in the different culture media (Table 1) at an optical density determined on a Cole Parmer 2800 UV/Vis Spectrophotometer at 600nm (OD600), corresponding to McFarland nephelometric scale 1 ($3.0x10^8$ CFU/mL) for a final volume of 50mL in Erlenmeyer.

Table 1
Composition of culture media

Constituents	MS1	FRU	HS	Z	Y
Glucose (g/L)	20	-	20	40	
Sucrose (g/L)	40	-	-		50
Fructose (g/L)	-	60	-		
Corn Steep Liquor (mL/L)				20	
Ethanol (mL/L)	50	50	-		
Yeast Extract (g/L)	5.6	5.6	5		5
Peptone (g/L)	-	-	5		
Citric Acid (g/L)	-	-	1.15		
Na_2HPO_4(g/L)	-	-	2.7	-	
KH_2PO_4(g/L)	1	-	-	2	3
$(NH_4)_2SO_4$(g/L)				4	5
$MgSO_4$ $7H_2O$ (g/L)				0.4	0.05

The bacterial suspensions were kept under constant agitation at 130rpm at 25ºC using Shaker Kasvi (Fig. 1), until the formation of SBC. The SBC produced were washed in distilled water and immersed in NaOH in a water bath at 65º C for 1 hour. After this procedure, they were washed in distilled water, with constant water change until reaching neutral pH. Part of the spheres produced were dried in a drying oven at 65°C and part of the spheres were lyophilized and taken for characterization by SEM, FTIR and TGA/DTG. The lyophilized SBC were used in the RIF release assays. The experiment was carried out in triplicate.

2.2.2. Characterization of SBC

The characterizations of the SBC obtained were performed by Fourier transform infrared spectroscopy (FTIR) to evaluate the main functional groups, using the Agilent Cary 630 FTIR spectrometer, in transmittance mode in the region from 4000 to 600cm^{-1}, and by Field emission gun-scanning electron microscopy (FEG-SEM), for analysis of the morphological characteristics in relation to the differences between the entanglements, fiber thicknesses and porosity, using the JEOL JSM-6360 LV microscope, after coating with carbon. The SBC obtained were also characterized by thermogravimetric analysis (TGA) and by derivative thermogravimetry (DTG). The curves were obtained using the TA Instruments SDT q600 equipment. The conditions used were oxygen atmosphere with continuous flow of 100 mL·min^{-1} and heating rate of 10°C·min^{-1}. X-ray diffraction (XRD) data were acquired in a XRD7000 Shimadzu diffractometer using CuKα radiation (λ = 1.5418 Å). The 2θ ranged from 5.0° to 60° with step scan 0.02° and speed scan 2°/min. The crystallinity index of BC membranes was determined by Segal et al., (1959) using the Eq. 1.

$$Crl(\%) = \frac{I_{200} - I_{am}}{I_{200}}.100$$

(1)

where *Crl* corresponds the crystallinity index, I_{200} is the maximum intensity of the (200) plane diffraction and *Iam* the intensity of the amorphous halo at 2θ = 18°.

2.2.3. Determination of volume/area ratio of SBC

To determine the swelling mass (Sm), lyophilized SBC produced in the different culture media were measured to determine the approximate volume of SBC (V_{SBC}) using the Eq. 2.

$$V_{SBC} = \frac{4..r^3}{3}$$

(2)

where V_{SBC} corresponds to the approximate volume of SBC, π to the number 3.1416 and *r* to the SBC radius (mm).

Triplicates of each SBC were chosen to determine the volume and the arithmetic mean was considered to calculate the volume of RIF solution to be used for impregnation of the SBC.

2.2.4. Sustained release capacity of SBCs analysis

For the test to evaluate the sustained release capacity of the antimicrobial drug RIF, the SBC obtained in the media of different compositions were impregnated with a volume (in µL) corresponding to half the volume, theoretically determined by Eq. (1), of the smallest SBC, with an aqueous RIF solution (stock 20µg·µL^{-1}). The SBC-RIF were placed in 24-well microplates kept under refrigeration at 4°C for 24 hours for complete RIF impregnation. After this procedure, the microplate containing the SBC was completely dehydrated in a ventilated oven (Novatécnica) at 35ºC, a temperature close to normal body temperature and, therefore, did not affect the stability of the RIF. Then, the SBC-RIF were applied to the surface of plates containing Muller-Hinton agar (MH) inoculated with *S. aureus* ATCC 25923. In contact with the surface of the MH plate, the SBC-RIF is swollen and initiates the release of the antibiotic. The plates were initially incubated in a bacteriological incubator at 36°C for 24 hours. After 24 hours of incubation, measurements of the bacterial growth inhibition zone were performed. Then, the SBC-RIF were removed from the plates and immediately transferred to new Petri plates containing MH agar, inoculated with *S. aureus* ATCC 25923, for maintenance of hydration and continuity of RIF release, and again incubated for 24 hours, to read 48 hours of release. The same procedure was repeated every 24 hours until there was no more inhibition zone. To carry out the test, the diffusion technique was used validated by the Clinical & Laboratory Standards Institute guideline (CLSI, 2019). The sustained release capacity was too analyzed by Franz cell release kinetics as described by Simon et al. (Simon et al., 2016). A 0.45um pore nitrocellulose membrane (NC) (MIllipore) was placed at the interface between the Franz cell owner and receiver compartments filled with 1 mL of phosphate buffer pH 7.4. On NC membrane, in the owner compartment, the different SBC-RIF were deposited. After 24 hours 1mL aliquots of the solution contained in the receiver compartment of the cell were collected and submitted to OD determination in UV-Vis 340nm every 24 hours, until complete drug release. After each analysis, the withdrawn content was completely returned to the receiver compartment of the Franz cell to enable the measurement of cumulative RIF release. The results were determined by comparison with a previously determined calibration curve.

3. Results And Discussion

3.1. Production of SBC in different culture media

As can be seen in Fig. 2, SBC were obtained in triplicate cuts, in different culture media with different compositions, however, with different macroscopic characteristics. These results demonstrate that the difference in SBC production is not related to the *K. hansenii* ATCC 23769 strain used, as all cultures were produced from a single pre-inoculum, and under the same agitation conditions, demonstrating that the differences in morphology are related to the different compositions of the media.

3.2. Macroscope and FEG-SEM analysis of the SBC produced in different culture media

When the macroscopic characteristics of the lyophilized SBC are compared (Fig. 3), a significant macroscopic difference was observed between the size of the SBC produced in Z medium compared to those produced in other culture media Y, HS, MS1 and FRU.

The FEG-SEM comparative analysis SBC produced in different culture media showed significative difference in relation to fiber interlacing, thickness, arrangement, and pore size as can be observed in greater detail in Fig. 4. It is possible to verify that the variation of the carbon source provided surface morphological differences both related to the thickness and the intertwining of the cellulose fibers.

It is possible to verify that the variation of the carbon source provided microscopic surface morphological differences both related to the thickness and the interlacing of the cellulose fibers. As can be seen, the characteristics of thickness, fiber entanglement and porosity of spheres produced in FRU, Z and MS1 media produced SBC with greater porosity.

El-saied et al. (El-saied et al., 2007) demonstrated that a BC produced in static culture using corn steep liquor (CSL) and molasses as carbon sources showed a higher degree of crystallization compared to the use of carbon and nitrogen sources such as glucose, mannitol, yeast extract and peptone. Vasquez et al. (Vazquez et al., 2013) also reported that production values complementing media with CSL for nitrogen requirements showed be higher than those achieved by the HS standard culture medium.

Costa et al. (Costa et al., 2017) evaluated the production of BC, in static condition, used Hestrin–Schm (HS) medium with different nutrient sources (glucose, yeast extract, peptone, Na_2HPO_4, and citric acid), and alternative medium (glucose, CSL, Na_2HPO_4, and citric acid). The alternative medium formulated with CSL led to the highest yield in terms of dry and hydrated mass, with the BC pellicles demonstrating a high concentration of microfibrils and nanofibrils forming a homogenous, compact, and three-dimensional structure. These results demonstrated that the nutrient source can be influence the properties of BC membrane.

According to Sperotto et al. (Sperotto et al., 2021) the optimal growth and performance of a bacterial culture is most influenced by the design and composition of its culture medium. According to these authors, CSL also contributes to the bioprocess economy, by replacing more expensive raw materials such as yeast extract.

The results obtained in the present work reinforce the influence of nutrient sources on the structural characteristics of BC in agitated cultivation.

Table 2
Results of the means and standard deviation of the SBC produced in different culture media.

Culture media	Volume		Standard deviation
	mm^3	µL	
FRU	22.5	22.5	0.098
HS	26.5	26.5	0.10
MS1	31.7	31.7	0.16
Y	21.5	21.5	0.12
Z	53.1	53.1	0.14

However, the internal volume of 'the SBC produced in the HS and Y media appears to be reduced, in relation to the FRU, Z and MS1 media, as they present, as can be seen in Fig. 4, greater density and degrees of interlacing of the fibers and lower porosity, being smaller, that presented by Y (Fig. 4d) and the one produced in the FRU medium is the one with the lowest degree fiber intertwining and greater porosity (Fi4 4a). and, therefore, greater internal volume, which reinforces the results obtained in the Franz cell diffusion and kinetics test.

3.3. SBC characterization by FTIR and XRD

The results obtained by the FTIR analysis showed characteristic bands of bacterial cellulose, with the interval of 3350-3500 cm^{-1} attributed to the O-H stretch, the interval 2800-2900 cm^{-1} attributed to the C-H stretches, the interval 1160 cm^{-1} attributed to the stretch C-O-C, while the 1035-1060 cm^{-1} range is due to C-O stretch. The FTIR spectra of the SBC shown in Fig. 5a confirm the purity of the SBC and reinforce the results obtained by the TGA/DTG (Fig. 6), indicating that a media with different compositions do not alter the chemical properties of pristine BC.

The result of the SBC XRD analysis showed that the composition of the media did not affect the BC crystallinity index, since its properties are influenced by the arrangement of molecules within the fibers. XRD demonstrated degrees of crystallinity with a typical BC profile (Fig. 5, Panel b). The main diffraction peaks were found at 2θ 14.7, 16.9 and 22.7 (Jung et al., 2010) and assigned to the 100, 010, and 110 crystal planes of cellulose Iα, respectively (Wahid et al., 2019). The degree of crystallinity ranged from 70 to 80%, and it was not possible to establish a relationship between the different culture media with the degree of crystallinity.

3.4. Characterization of SBC by TGA/DTG

As in Fig. 6, the variation in the mass loss of the SBC was negligible, and it is also possible to assess that there were no inorganic residues because between the temperatures of 500°C and 550°C 100% of the

mass of the SBC was lost. This result reinforces that the use of culture media with different compositions does not alter the thermal behavior of the polymer and does not influence the purity of the SBC

3.5. SBC-RIF characterization by FTIR and FEG-SEM

Because no differences were detected in the FTIR spectra of the SBC, the produced in the FRU medium was chosen, for greater capacity for sustained release of RIF. Pristine SBC and SBC-RIF were subjected to comparative analysis. The Fig. 7 shows of the chemical structure of RIF and SBC, and possible interaction mechanisms (a) due to the fact that RIF has flexible backbone with two phenolic and two aliphatic OH groups in addition to nitrogen and oxygen donor atoms (Saad et al., 2020) which allows the interaction, through hydrogen bonds with SBC. The Fig. 7b show the and the results of the comparative analysis between the FTIR spectra of pristine SBC, the SBC-RIF and commercial RIF. As can be seen, the FTIR spectrum of the commercial sample of RIF used, similar to that published by Schianti et al. (Schianti et al., 2013), with SBC-RIF and pristine SBC demonstrating antibiotic incorporation.

The proposal interaction between RIF and SBC (Fig. 7a) can be confirmed by FEG-SEM (Fig. 8) of SBC-RIF, with SBC produced by *K. hansenii* ATCC 23769 in FRU and Z (highest concentration released/96 hours) and Y (shorter concentration released/96 hours). The Fig. 8 show surface distribution of the classical crystal structure of RIF, after water dissolution and dry, similar to that published by Agrawal et al. (Agrawal et al., 2004). The Figure show the crystals in the SBC-RIF produced in FRU (Panel a), Y (Panel b), and Z (Panel c) media.

As observed in the Fig. 8, the RIF characteristics were maintained two SBC, suggesting that the sustained release capacity is associated with the lower degree of interlacing and thickness of the fibers, and the greater porosity of the SBC produced in FRU and Z media, as can be observed in the Fig. 4 (Panels a and e) which allowed greater diffusion between the SBC fibers by RIF in solution, providing major retention after drying.

Thus, the results of this work demonstrate that a product with great possibility of being used as a support for a drug release was obtained, since SBC is a biomaterial known for its hypoallergenic, biocompatible, nanostructured characteristics. It can also be observed that, although the largest volume was of the membrane produced in the Z medium, the microscopic characteristics related to the degree of interlacing, thickness of the fibers, and the porosity were similar to those observed for the SBC produced in the HS (Fig. 4b), MS1 (Fig. 4c), being the SBC produced in the Y medium (Fig. 4d), with greater degree interlacing thickness fiber and less porosity determining a lower permeation of RIF between the fibers of SBC, produced in Y medium, promoting a higher surface concentration and, therefore, less interaction of the RIF with this SBC, that cause a release burst, justifying the lower release capacity by this device. Thus, the relationship can be made between the SBC produced in the FRU medium, which had lower fiber interlacing and thickness, but greater porosity, which determined substantial permeation of RIF in the pores and, consequently, large interaction with the SBC, which may justify the greater sustained release capability of this SBC. Another fact to be highlighted is that all SBCs obtained were swelled with the

same volume and concentration of RIF, but as can be seen, the volume of SBC produced in Z medium was significantly higher compared to the other SBCs, a fact that allows its swelling with a greater volume of RIF, which can increase the ability of sustained release of the antibiotic.

3.6. Sustained release capacity of SBC analysis

The data of the sustained release capacity of RIF by SBC were performed as described in item 2.2.4. All SBC were swollen with 10 µL of aqueous RIF solution (stock 20µg·µL).

The SBC produced in the different media produced in the different sustained release capacity, as observed by the comparative analysis of the growth inhibition zones every 24 hours presented in the Fig. 9 and Table 3.

All SBC produced in different culture media were swollen with the volume of the RIF aqueous solution corresponding to the volume (20µL), theoretically determined by Eq. (1), of the smallest SBC, which in this case was produced in the FRU medium. Thus, all SBC contained the same RIF mass (400µg), being the measure of the inhibition zones, related to fiber interlacing, thickness, arrangement, and pore size presented in the FEG-SEM comparative analysis (Fig. 2).

The characteristics of thickness, fiber entanglement and higher degree of porosity of SBC produced in FRU and Z and MS1 media determined greater sustained release capacity compared to SBC produced in HS and Y media.

Table 3
Sustained release of SBC-RIF by diffusion assay

Culture media	**Inhibition Zone (mm)**			
	24h	**48h**	**72h**	**96h**
FRU	25.17±1.72*	21.40±1.91*	21.28±1.29*	19.80±1.25*
HS	23.90±1.25*	21.60±1.13*	18.33±3,39*	16.94±2.77*
MS1	27.43±0.49*	21.21±2.31*	20.61±1.62*	18.51±1.45*
Y	25.13±2.35*	14.20±1.93*	14.32±1.63*	12.82±2.44*
Z	23.12±1.32*	22.94±1.51*	21.28±1.39*	19.18±1.15*
***** Standard deviation				

These results suggest that the composition of the culture media interfered directly with bacterial metabolism, a fact that promoted the alterations in the physical characteristics of BC, since the bacterial strain and the culture conditions were the same for the different culture media.

3.7. Sustained release capacity of SBC analysis by kinetics by Franz cells.

In view of the results obtained in the agar diffusion tests, the release kinetics assay was performed using Franz cells. The results of this assay are shown in Fig. 10.

As observed in Fig. 10, the SBC, produced in the different culture media presented dissimilar RIF release capacity. These results demonstrated that SBC produced, respectively, in FRU, Z and MS1were capable to maintained greater release concentration when compared with SBC produced in HS and Y media.

Drug release from different types of systems can occur by distinct mechanisms such as drug dissolution combined with diffusion through pores or membranes, polymer degradation and/or erosion and osmotic pumping (Carvalho et al., 2021; Fredenberg et al., 2011). Such mechanisms, in turn, can be controlled by gradient drug concentration, penetration of the dissolution drug into the system, or ionic strength swelling rates, among others (Kamaly et al., 2015).

The results of drug release tests, both by diffusion and by Franz cells, determined a high degree of swelling in the SBC, suggesting that the solubilization of RIF crystals gradually decreased the SBC-RIF interaction, promoting its sustained release (Fig. 11).

4. Conclusion

SBC, with high reproducibility, were obtained in cultures under constant agitation using media with different types and concentrations of carbon sources. The SBC obtained showed different characteristics in relation to thickness, fiber entanglement and degree of porosity, since the bacterial strain and culture conditions were the same for the different culture media. The results suggest that the composition of the culture media may interfere with the biosynthetic metabolism of biopolymer synthesis, leading to the production of SBC with distinct macro and microscopic physical characteristics, The characteristics of interlacing and thickness fiber and higher degree of porosity of SBC produced in FRU and Z and MS1 media, respectively, determined greater RIF sustained release capacity when compared to SBC produced in HS and Y media (lower capacity). Thus, the results of this work demonstrate that a product with great possibility of being used as a support for drug release was obtained, since SBC is a biomaterial known for its hypoallergenic, biocompatible, nanostructured characteristics. In this way, SBC will be able to contribute to the design of new drug delivery systems with biomedical applications.

Declarations

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Conflict of interest: The authors declare that they have no conflict of interest.

Ethical approval: All ethical rules were obeyed; no human or animal experiments were performed.

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Figures

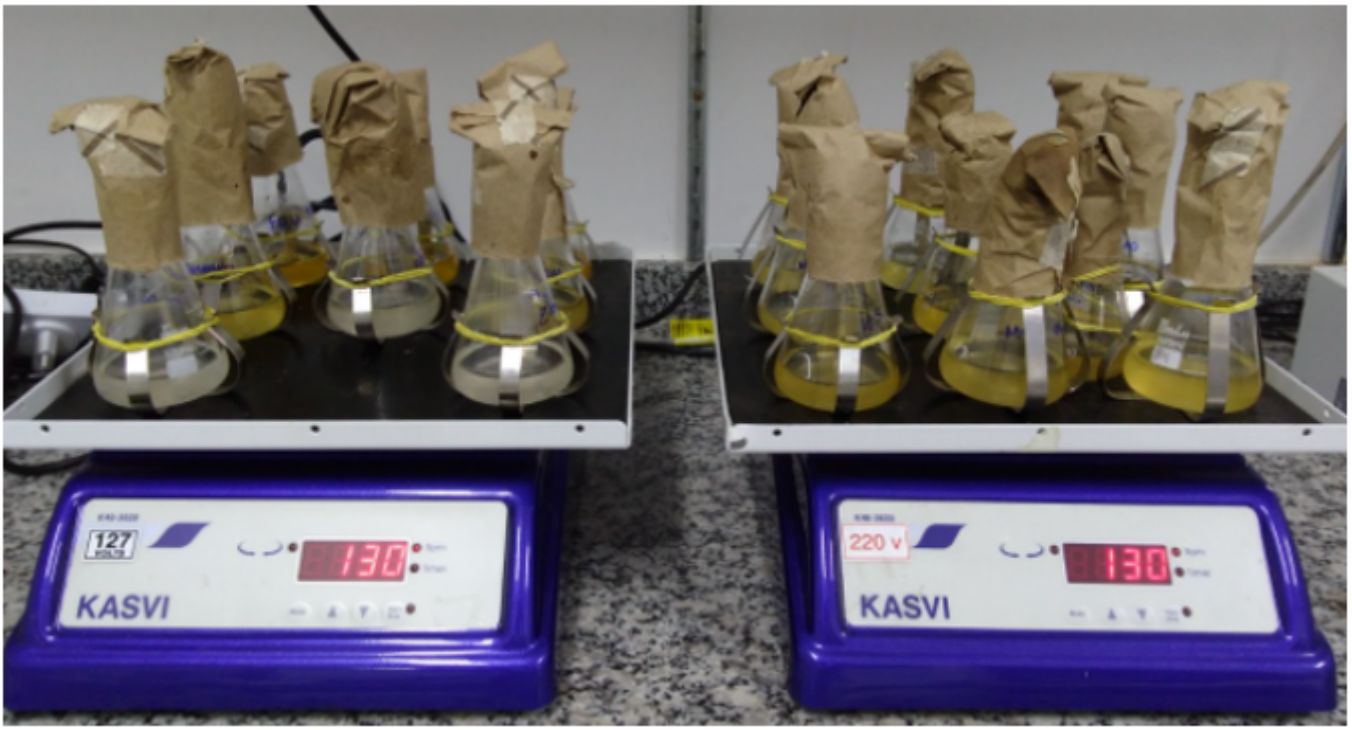

Figure 1

SBC production system at room temperature of 25°C under agitation at 130rpm, in different culture media.

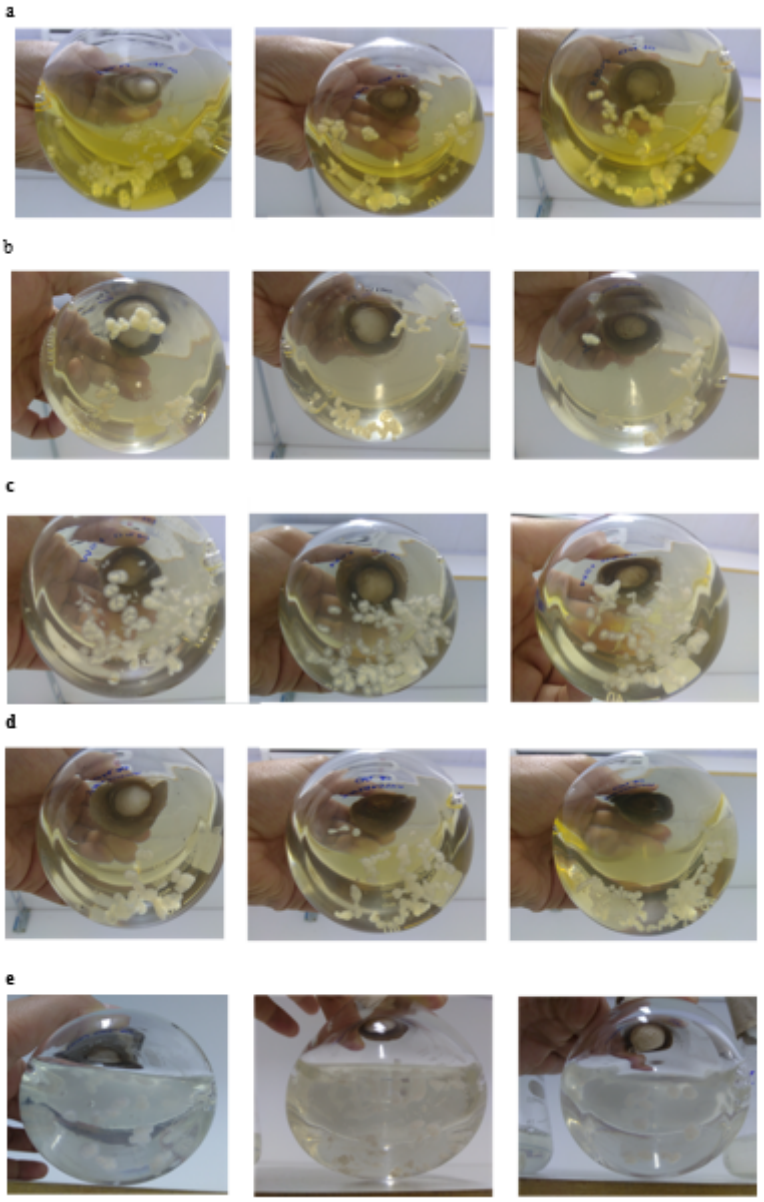

Figure 2

SBC produced, in triplicate, in different culture media. Panel a: FRU; Panel b: HS; Panel c: MS1; Panel d: Y; Panel e: Z.

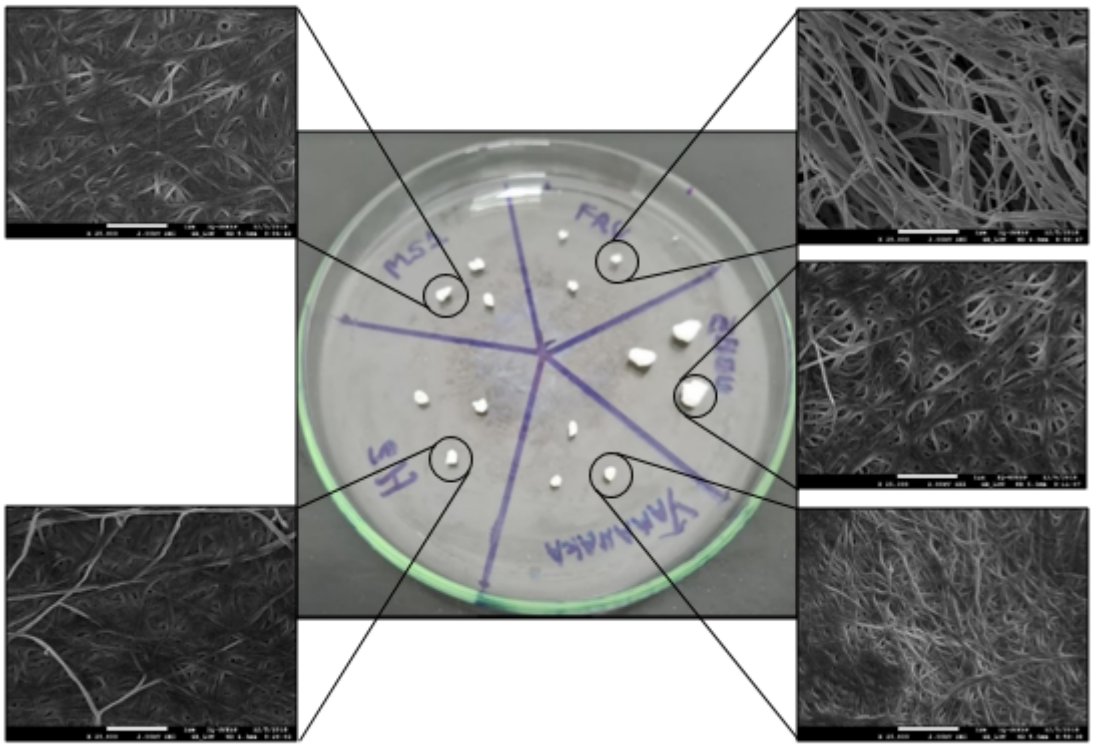

Figure 3

Macroscopic and correspondent microscopic characteristics of the SBC produced in different culture media in triplicate

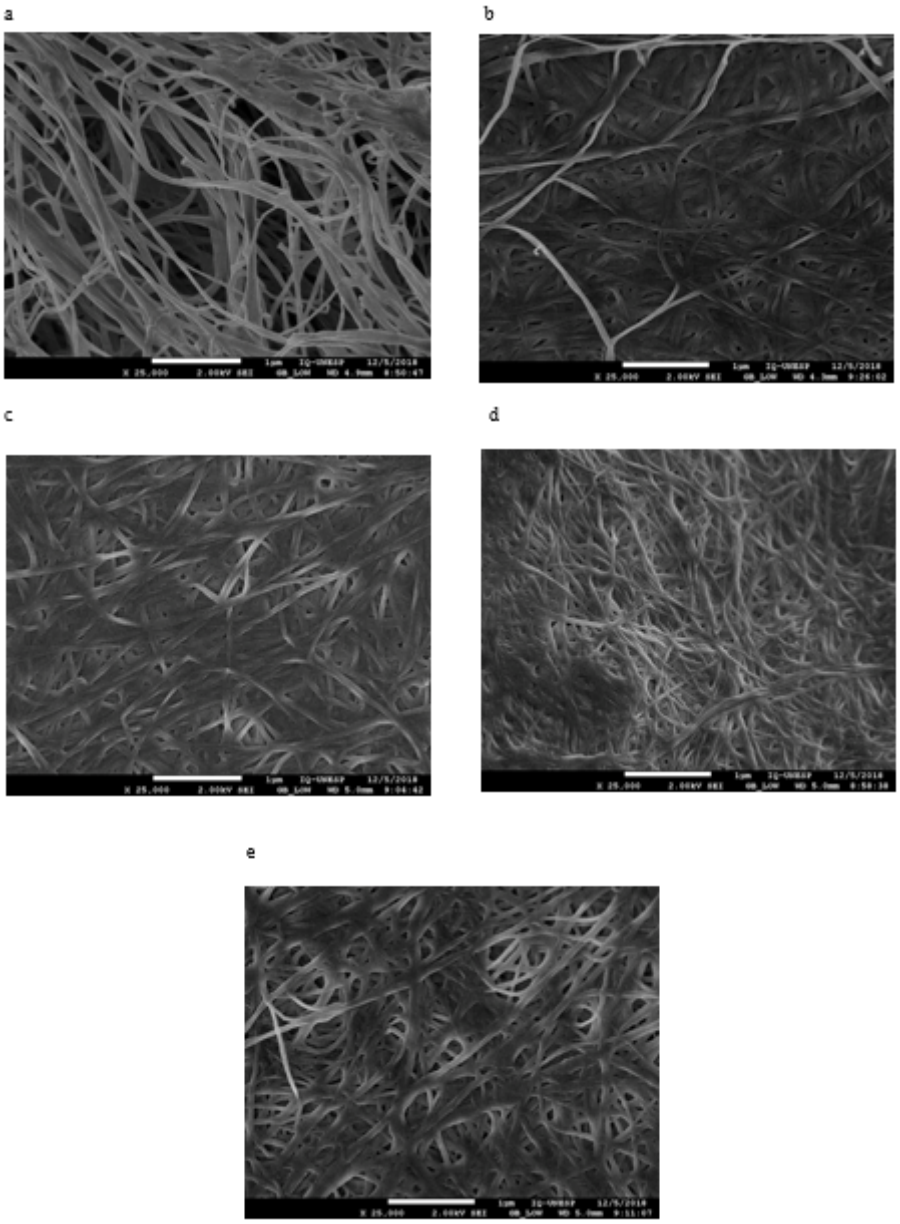

Figure 4

FEG-MEV (25,000x) of the SBC produced. Panel a: FRU; Panel b: HS; Panel c: MS1; Panel d: Y; Panel e: Z.

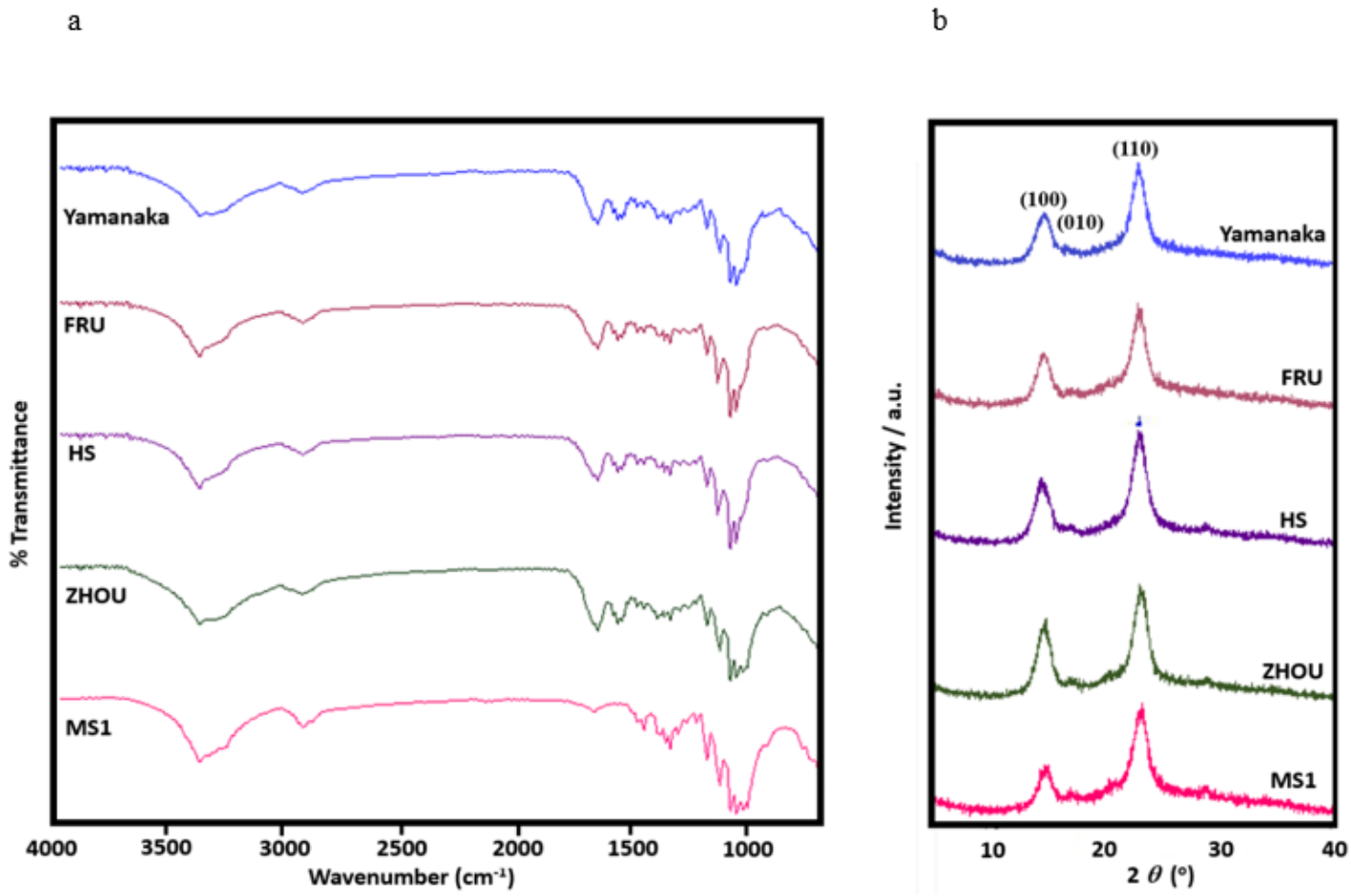

Figure 5

FTIR spectra (a) and XRD (b) of SBC beads produced by *K. hansenii* ATCC 23769 in media containing different compositions.

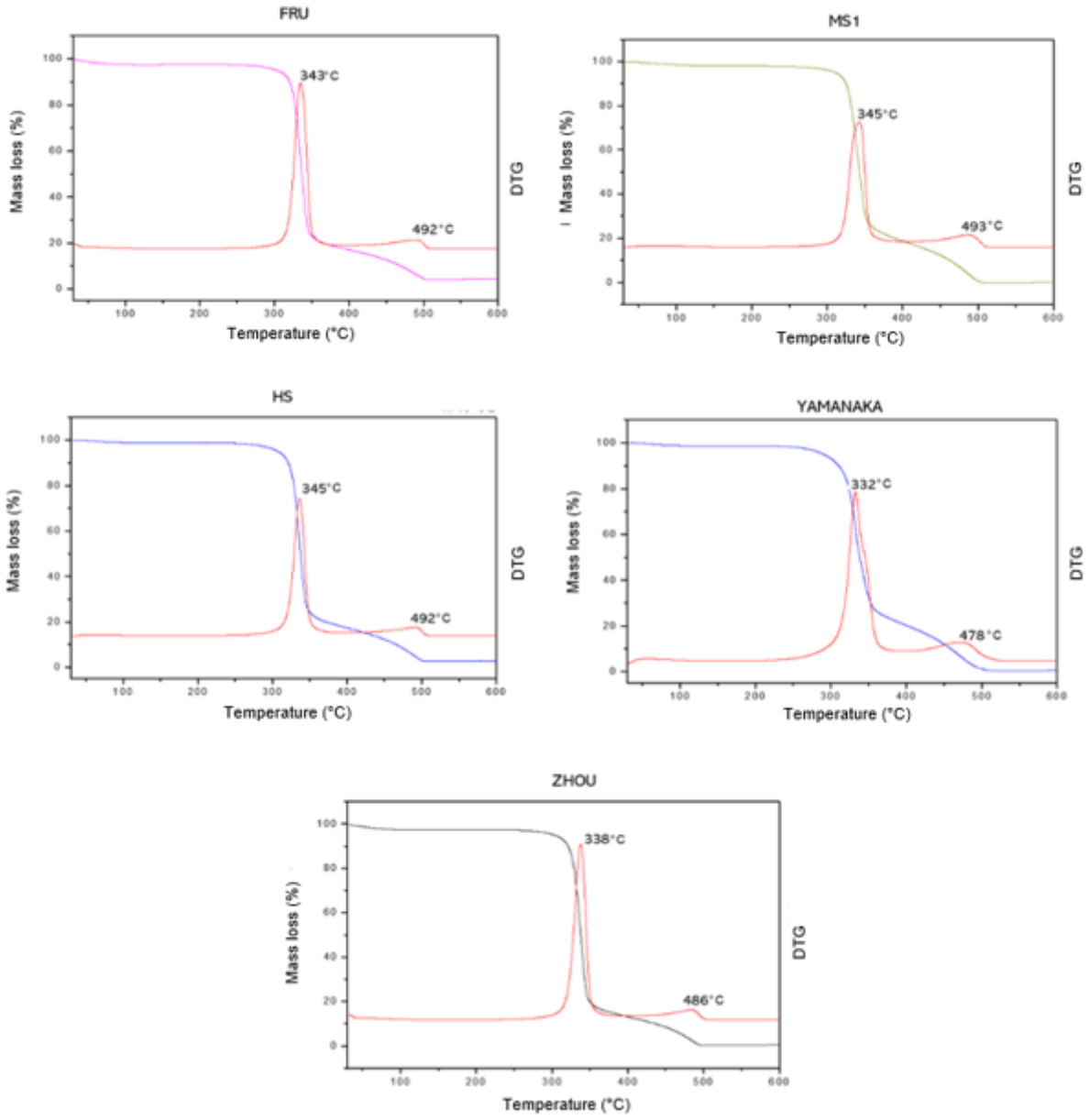

Figure 6

TGA/DTG of SBC produced by *K. hansenii* ATCC 23769 in media with different compositions

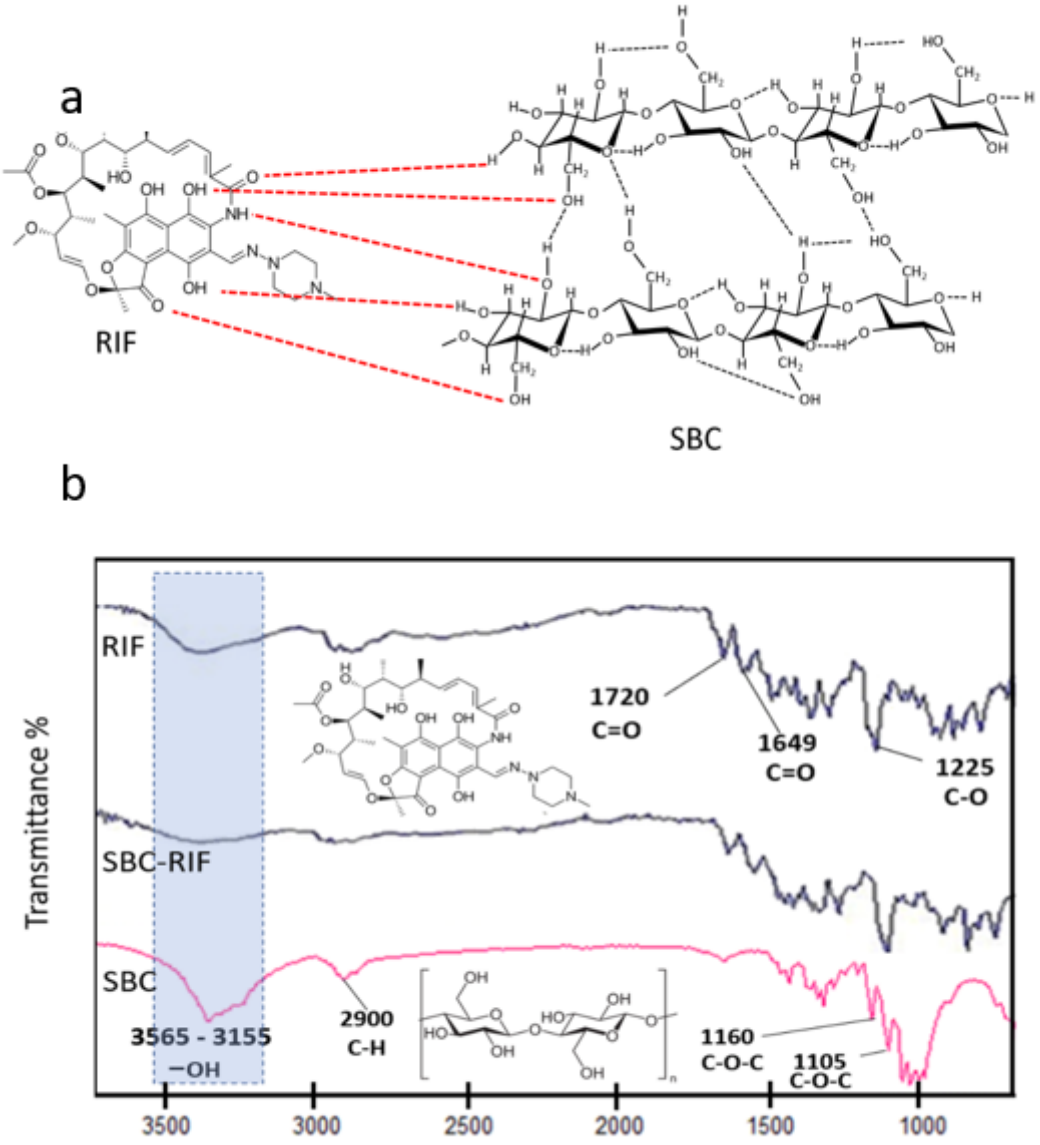

Figure 7

Schematic representation of the chemical structure of RIF, SBC, and possible hydrogen bond interactions between them (a), and FTIR spectra of RIF, SBC-RIF, and SBC (b).

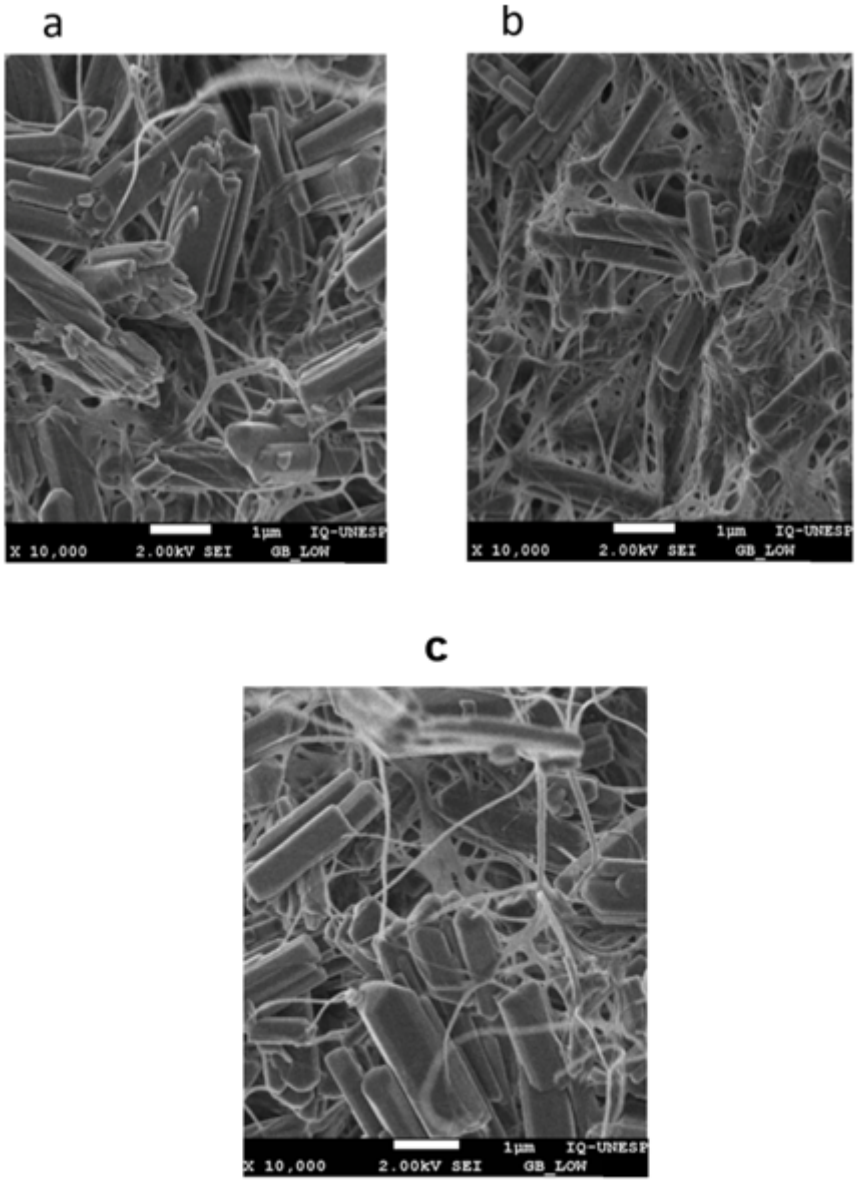

Figure 8

FEG-SEM of SBC-RIF, with SBC produced by *K. hansenii* ATCC 23769 in FRU and Y culture media.

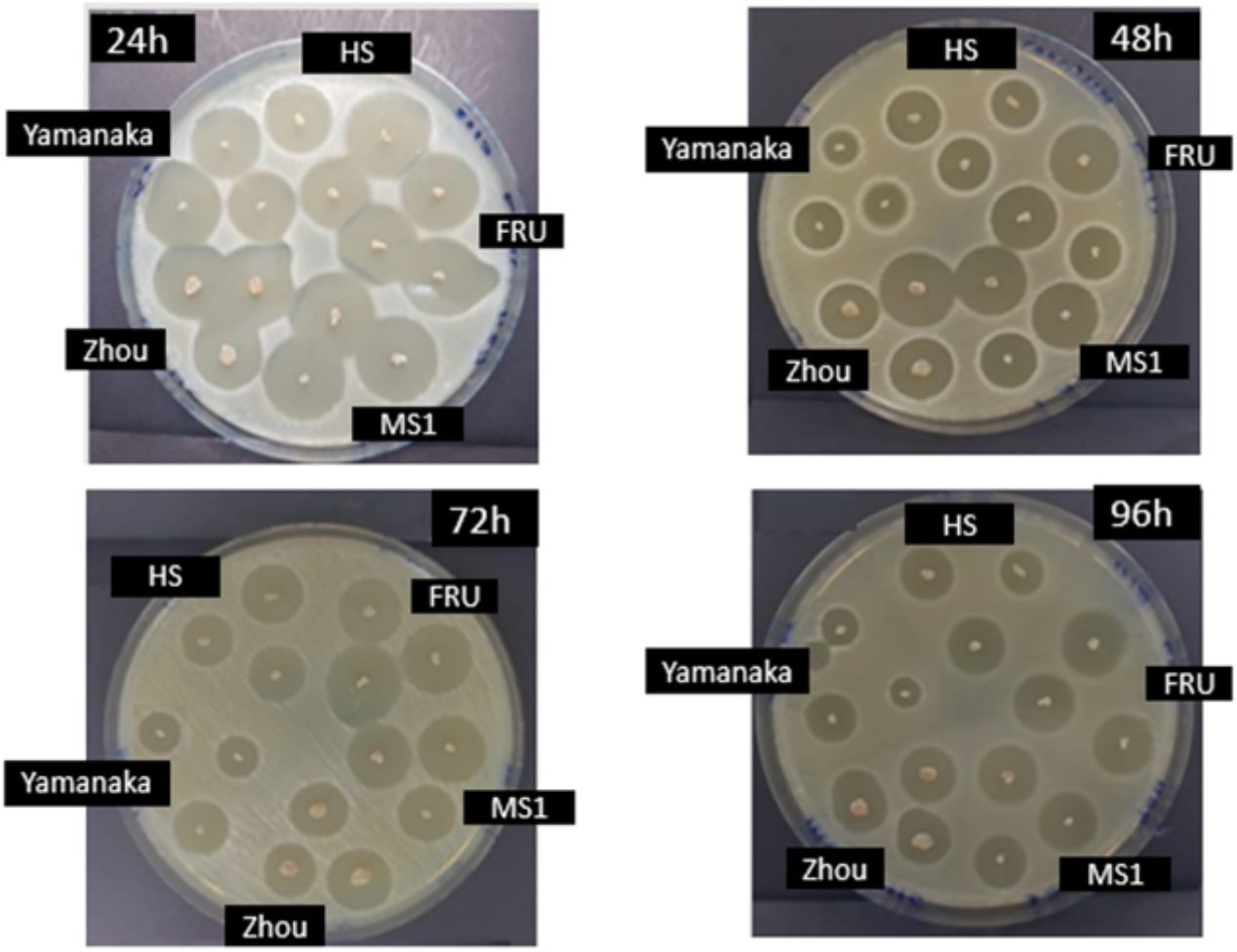

Figure 9

Results of RIF sustained release assay by SBC produced in different culture media.

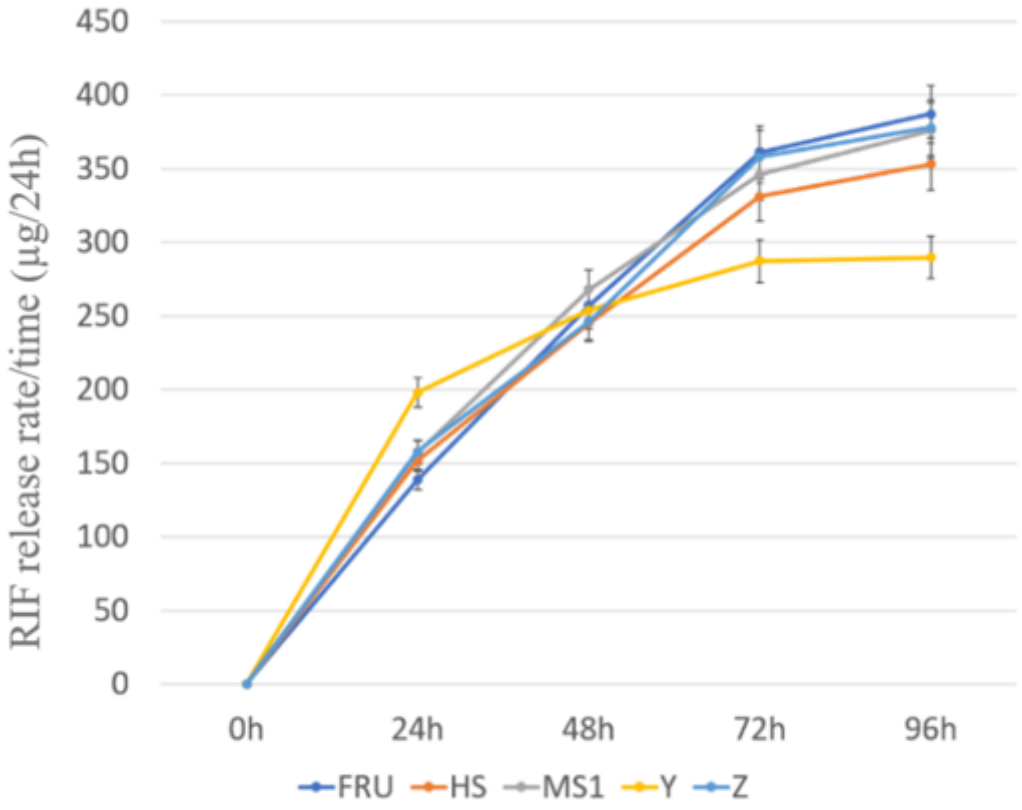

Figure 10

RIF release rate/time by Franz cell kinetics assay

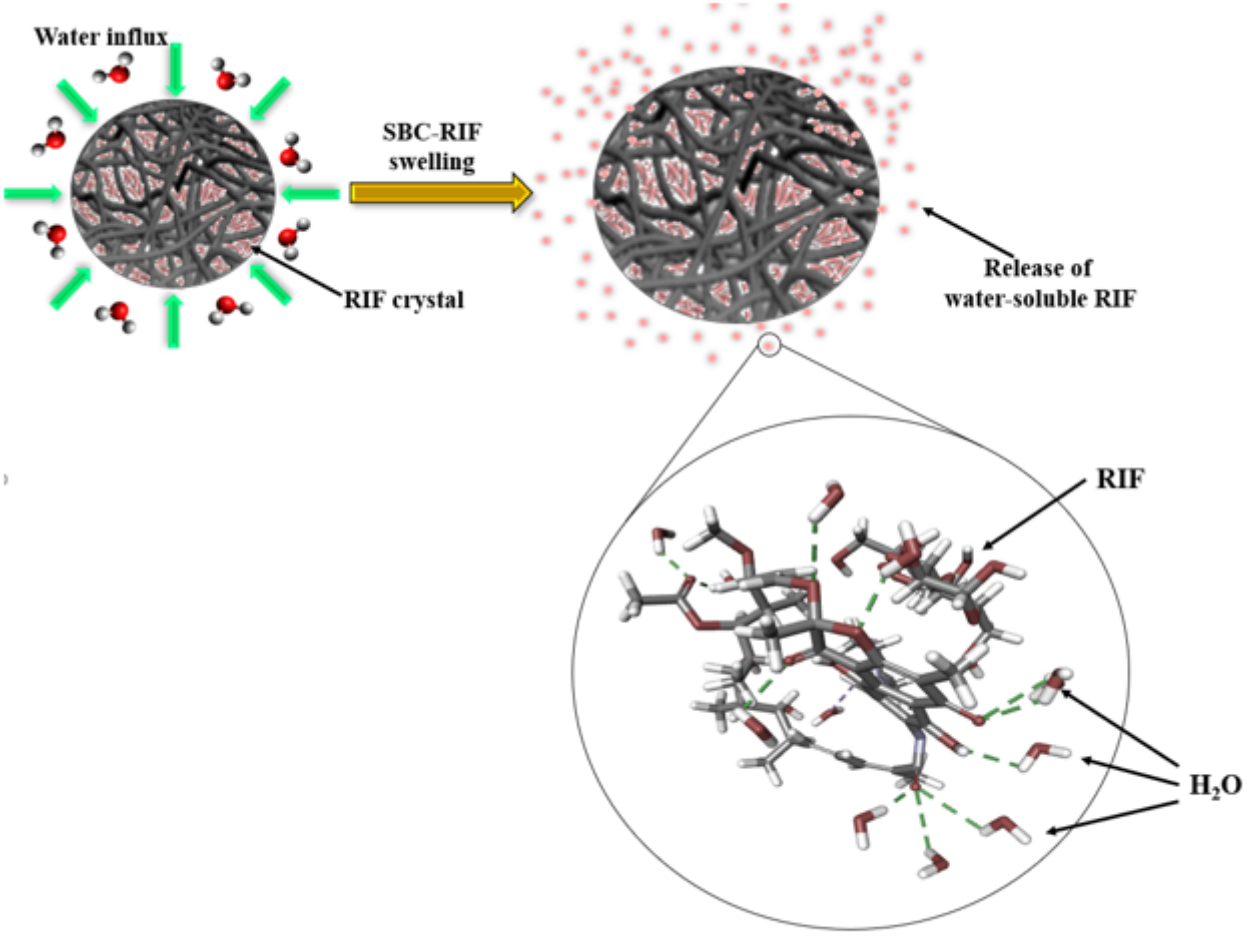

Figure 11

Proposed mechanism for sustained release of water-soluble RIF from RIF-SBC after swelling.