Annals of Warsaw University of Life Sciences - SGGW
Forestry and Wood Technology № 117, 2022: 35-43
(Ann. WULS - SGGW, For. and Wood Technol. 117, 2022: 35-43)

The influence of microbiological medium composition on the efficiency of bacterial cellulose synthesis

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Abstract: *The influence of the composition of microbiological medium on the efficiency of bacterial cellulose synthesis.* The main objective was to investigate the effect of culture medium composition on the process of bacterial cellulose synthesis. Five different nutrients were used as carbon sources for cellulose synthesising microorganisms: glucose, fructose, erythrol, inulin and lactose, added to the medium at three different concentrations (1%, 2.5%, and 4.5%). It was observed that the type and amount of nutrients included in the culture medium significantly affected the cellulose synthesis efficiency. It was observed that the best results of polymer synthesis were obtained on medium containing 1% fructose. Furthermore, the results obtained clearly confirm that the composition of the culture medium has a significant effect on the water retention of the polymer during its synthesis on the culture media.

Keywords: bacterial cellulose, SCOBY microorganisms, medium composition

INTRODUCTION

Nowadays, with the progress of civilisation, the demand for raw materials is increasing. One of them is wood. Increasing demand and limited amount of raw material cause that more and more emphasis is put on production of wood-based materials and their modifications (Wacikowski et al. 2020). Increasing demand for cellulose has led to increased felling of trees for its extraction, which causes the adverse phenomenon of deforestation (Park et al. 2003). Bacterial cellulose may be a rescue in this situation. Cellulose itself is the most abundant biopolymer on the planet. It is found in all plants. Furthermore, it is produced by microorganisms such as Acetobacter xylinum or Gluconacotobacter xylinus as a product of the metabolism of glucose and other sugars (Cannon et al. 2008).

Bacterial cellulose is an exopolysaccharide. It is composed of b-1,4-D-glucopyranose units (Zhang et al. 2018). Bacterial ceulose is produced by acetic acid bacteria through oxidative fermentation (EL-Saied et al. 2004). Compared to other polymers, bacterial cellulose is characterised by high purity and does not require extraction moreover, it is characterised by high biocompatibility which makes it suitable for medical engineering (Torres et al. 2012). Bacterial cellulose is a homopolymer with a rigid chain. Its main advantage is its hydrophilicity, which makes it susceptible to chemical modification (Moon et al. 2010). Bacterial cellulose is in the form of nanofibres about 100 μm long and 100 nm in diameter. Moreover, its fibres are 100 times thinner than those of other biopolymers (Stanislawska 2016), but this does not affect its durability. All these aspects make bacterial cellulose a very interesting and promising material for use in many industries (Gregory et al. 2021). Bacterial cellulose is used in the medical field, electrical engineering or paper industry, among others. Currently, however, the demand for nanomaterials is so high that various modifications are used to create composites containing bacterial cellulose in order to improve the properties of the material (Esa et.al. 2014). The extremely good physical and mechanical properties of bacterial cellulose also make it a potential material for use in the production of wood-based materials (Nugroho et al. 2021).

The degree of polymerisation of bacterial cellulose averages between 9 000 and 20 000 (Tabuchi et al. 1998). It has excellent mechanical properties such as tensile modulus and

elasticity, which are about 16.9 GPa and about 2 GPa, respectively (Gallegos et al. 2016). These values change with changes in the culture medium. It has a significant influence on the cellulose properties. It has been shown that changes in substrate composition (e.g. addition of peptone to sucrose-containing substrate) affect the thickness of the obtained films, their elongation and tensile strength (Betlej et al. 2020). Moreover, an additional advantage is that bacterial cellulose is fully eco-friendly and recyclable and is easy to decompose. As is well known nowadays, this issue is extremely important and the search for and use of environmentally safe biomaterials is widely promoted worldwide (Lu et al. 2014; Ludwicka et al. 2019).

Unfortunately, mass production of bacterial cellulose is quite problematic due to low production yields and relatively high costs. For this reason, despite its excellent properties, its application is limited. Hence, it is extremely important to find solutions to these problems and to search for, for example, a carbon source with a shorter fermentation process (Esa et al. 2014). In order to increase the efficiency of cellulose production, research is being conducted on the composition of the substrate, which plays a huge role in the cellulose synthesis process. Nutrients such as carbon or nitrogen are used, but also additives in the form of metal ions and organic substances (Tingfen et al. 2019). Another factor that enhances the cellulose synthesis process is the use of additives in the form of acetic acid, controlling the amount of dissolved oxygen and maintaining an optimal pH of the medium. These are achieved by an appropriate selection of the proportions of the components, which are fructose, glucose and the aforementioned acetic acid (Vandamme et al. 1997). Furthermore, there is evidence that cellulose production can be increased by the addition of water-insoluble microparticles such as diatomaceous earth, clay particles, silica and glass sand (Vandamme et al. 1997). Yeast extract, which is a rich source of nitrogen, is also an additive used. In addition to glucose and fructose, oligo- and polysaccharides are used as carbon sources (Krystynowicz et al. 2002).

Data from the researchers clearly confirm that the composition of the nutrient solution determines the properties of cellulose. Sang et al. (2010) proved that changing the carbon source affects the cellulose synthesis process and its yield. The most commonly used types of medium are fructose and glucose. Studies show that the yield of cellulose synthesis grown on fructose medium is higher than when glucose is used as a carbon source (Keshk et al. 2005). However, these parameters can be modified by adding various peptide- and vitamin-rich components to the medium, such as yeast extract, which positively influence the synthesis process by significantly improving it (Çoban et al. 2011). Many sources report that the highest synthesis yield is obtained for glycerol (Keshk et al. 2005; Zikmanis et al. 2021).

This paper presents an evaluation of the effect of different nutrient substrates in the growth medium of microorganisms forming an ecosystem called SCOBY on the efficiency of cellulose synthesis and the amount of polymer obtained. A research hypothesis was formulated in the paper: "the composition of the culture medium determines the processes related to the efficiency of cellulose synthesis and the water content of the polymer".

MATERIALS AND METHODS

Bacterial cellulose was obtained during the culture of microorganisms forming an ecosystem called SCOBY on semi-synthetic media. Five different types of media were prepared to compare the efficiency of cellulose synthesis in different culture environments. The prepared media contained 0.01% peptone as a source of nitrogen. Additionally, saccharide components and their derivatives were used as carbon sources. The list of applied culture medium components is presented in Table 1.

Table 1. Composition of the culture medium in the culture of microorganisms forming the ecosystem called SCOBY

Substrate No.	Carbon source		Peptone - nitrogen source Quantity (w/v)
	Type of ingredient	Quantity (w/v)	
A1	Glucose	1%	0.01%
A2		2.5%	
A3		4.5%	
B1	Fructose	1%	0.01%
B2		2.5%	
B3		4.5%	
C1	Erythrol	1%	0.01%
C2		2.5%	
C3		4.5%	
D1	Lactose	1%	0.01%
D2		2.5%	
D3		4.5%	
E1	Inulin	1%	0.01%
E2		2.5%	
E3		4.5%	

The nutrients needed for the study were dissolved in distilled water. The solution thus obtained was sterilised in a steam autoclave at 121°C for 20 min. The next step was to apply 1 ml of SCOBY microorganisms starter culture homogenate on the prepared sterile medium. The microbial culture process was carried out in a thermal incubator and its duration was 14 days. The temperature and relative humidity conditions of the culture conditions were 26±2°C and 66±2%, respectively. After the designated culture time, the cellulose produced was removed from the surface, which was dried on tissue paper and then weighed. The cellulose obtained was washed 3 times in distilled water for 12 h. The cleaned cellulose was subjected to an oven drying process at 30±2°C until the mass of cellulose was unchanged. After the process, the percentage yield of cellulose synthesis was determined according to the guidelines developed by Sharma and Behardwaj (2019) where:

percentage of synthesis [%] = (dry mass of polymer formed [g]/sum of the initial concentration of sucrose and additional component [g]) x 100

Meanwhile, the dry mass of the polymer produced was determined from the formula:

dry mass of polymer formed = mass of dried polymer [g]/volume of medium [l]

The water retention value (WRV) in bacterial cellulose was determined as the ratio of the mass of water retained in the cellulose after removal from culture to the mass of polymer dried to constant weight under given temperature conditions. The water retention value represents the amount of water that a bacterial cellulose sample is able to absorb. The water

retention value was determined according to the assumptions given by Sharma and Behardwaj (2019), according to the formula:

$$\mathrm{WRV} = \left(\frac{\mathrm{m1}}{\mathrm{m2}} - 1\right) \mathrm{x100}$$

where,
m1- wet mass [g] of cellulose
m2- mass [g] of dry cellulose

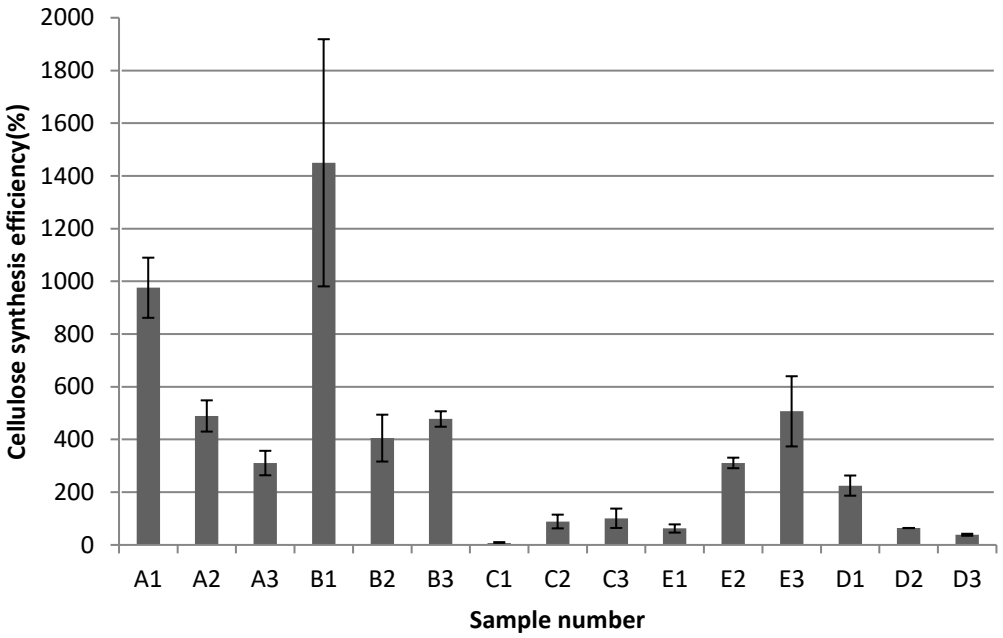
Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics 23.0.0.2 software. Two-factor analysis of variance (ANOVA) was used. The significance of the hypothesis was tested using the Turkey test. The correlation test determined the relationship between the variables.

RESULTS

Cellulose synthesis efficiency varied with the change in the type and amount of nutrient. The study shows that there are statistically significant differences between the nutrient content of the substrate and the cellulose synthesis efficiency (Table 2).

Figure 1. Variation in cellulose synthesis yield as a function of culture medium composition.



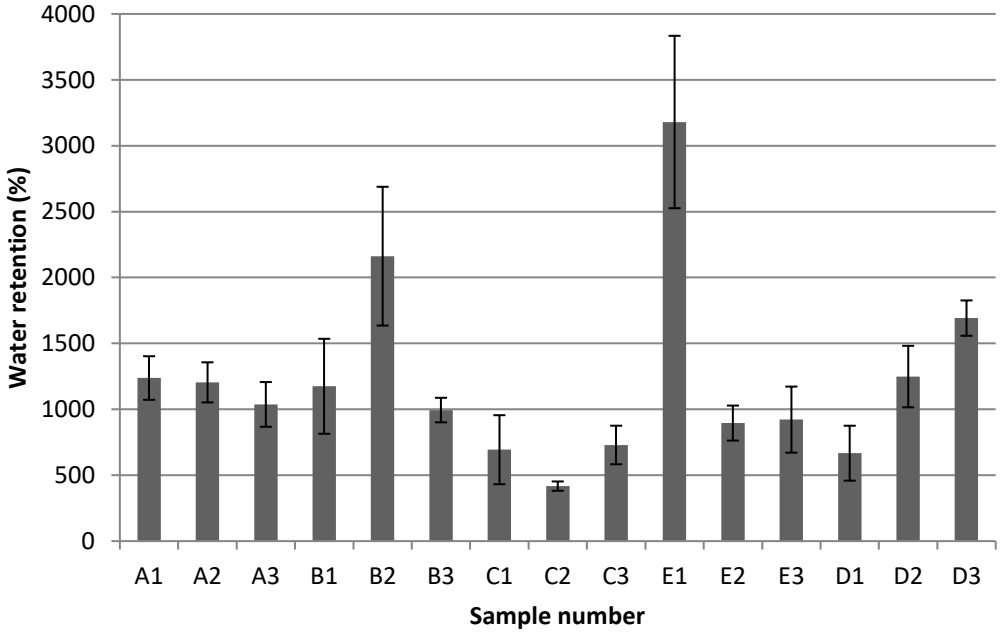
The highest cellulose synthesis efficiency was obtained for the medium containing fructose.(Fig.1) At a fructose content of 1% in the growth medium, the efficiency of cellulose synthesis by microorganisms was as high as 1449.6%. However, increasing the amount of monosaccharide in the medium clearly inhibited the polymer synthesis efficiency. Good cellulose synthase yield results were also obtained for low concentrations of glucose. Here, too, it was observed that at higher glucose concentrations the polymer synthesis yield was markedly reduced.

Similar results were obtained in other studies (Embuscado et al. 1994). The authors also achieved the highest cellulose synthesis efficiency in a culture medium containing fructose and

fructose with sucrose. The authors of this study also found that on the medium containing glucose, the cellulose synthesis yield was lower compared to the synthesis yield obtained on the medium with fructose. The lowest cellulose synthesis efficiency was observed in samples with erythrole and lactose as carbon sources. Similar results were obtained in a study by Kiselyova et al. 2021, which showed that cellulose production was significantly more efficient on glucose and fructose substrates and poorly on those containing lactose. Slightly higher polymer synthesis efficiency was obtained on the medium containing inulin as a carbon source. In addition, it was observed that the best cellulose synthesis efficiency was obtained when inulin was added to the growth medium at 4.5%.

The results obtained show that differences in the quality and quantity of nutrients have a statistically significant effect on water retention in cellulose samples. The highest retention was observed in samples containing inulin. In this case, with increasing polysaccharide content in the growth medium of microorganisms, water retention in synthesised cellulose decreased (Fig. 2). The lowest water retention was obtained in cellulose samples synthesised on erythrole medium. In the case of cellulose obtained on glucose medium, it was noted that water retention in these samples is at a similar level. Studies by other researchers confirm that bacterial cellulose samples obtained on glucose and fructose media have relatively high water retention rates (Rozenberg et al. 2016).

Figure 2. Water retention in cellulose synthesis as a function of culture medium composition.



In the case of the moisture content tests, the discrepancies were relatively small. The highest moisture content was found in the cellulose sample obtained on a substrate containing 1% inulin, while the lowest moisture content was found in the polymer sample obtained on a substrate containing 2.5% erythrol (Fig. 3). Moisture contents for all samples were almost identical. The smallest discrepancies were observed in the glucose samples.

Figure 3. Moisture assessment of cellulose obtained from different culture media.

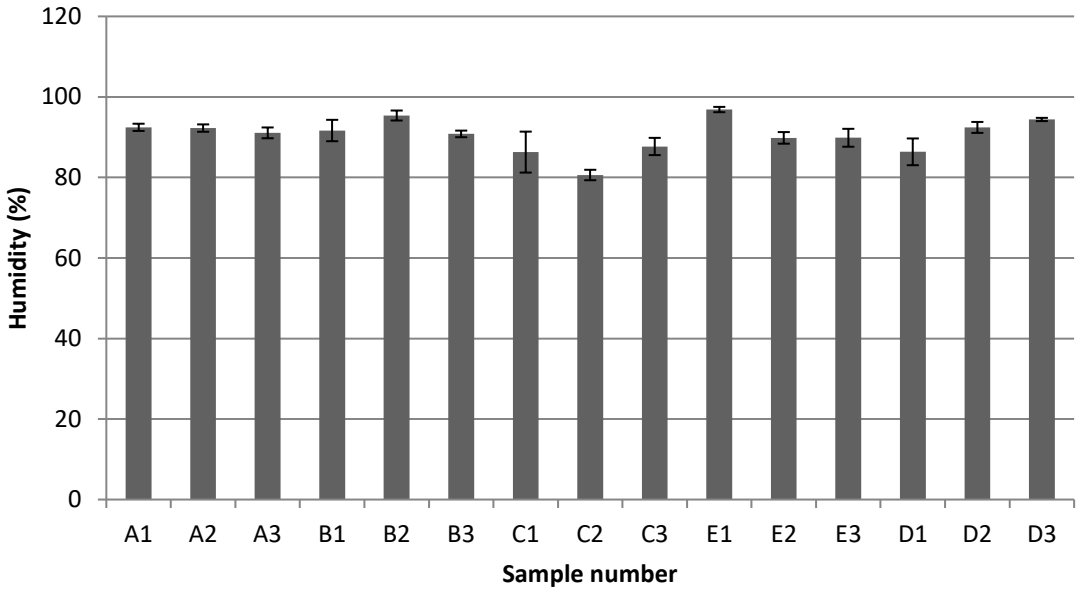


Table 2. The relationship between the examined bacterial cellulose properties and the composition of the culture medium

Specification	Amount of sugar (%)	Type					Statistical significance		
		Glucose	Fructose	Erythrol	Inulin	Lactose	P	R	PxR
Synthesis efficiency	1	975.6667 ±114.11544	1449.6667 ±468.87561	6.7767 ±3.81294	62.3330 ±15.62050	225.0000 ±38.31449	*	*	*
	2.5	489.2200 ±59.25273	405.2233 ±88.86703	88.7777 ±26.08606	311.0000 ±19.95274	64.0000 ±0.33300			
	4.5	310.6133 ±46.43425	477.6667 ±29.30925	101.1113 ±36.72785	506.7780 ±133.13934	38.5553 ±3.75044			
Water retention	1	1237.5633 ±165.32206	1174.7067 ±360.33090	693.5267 ±261.86695	3180.0900 ±654.06061	667.0567 ±208.47499	*	*	*
	2.5	1204.3867 ±152.27331	2161.9300 ±526.42333	417.1800 ±35.37184	895.5600 ±132.83715	1248.3233 ±233.17608			
	4.5	1037.1467 ±169.63359	994.2033 ±93.09531	729.3400 ±146.39214	921.8133 ±250.73787	1692.1733 ±134.12127			
Humidity	1	92.4500 ±0.89560	91.6587 ±2.65494	86.2940 ±5.08814	96.8643 ±0.65841	86.3637 ±3.32397	*	*	X= 0.625
	2.5	92.2627 ±0.91374	95.3867 ±1.23873	80.6053 ±1.29555	89.8270 ±1.44166	92.4270 ±1.36146			
	4.5	91.0733 ±1.34075	90.8143 ±0.82217	87.6917 ±2.13900	89.8500 ±2.22719	94.4000 ±0.40245			

*- statistically significant effect ($p<0.05$)

X- effect not statistically significant ($p>0.05$)
P- % sugar content in the substrate
R- type of sugar

Table 3. Correlation between selected properties of bacterial cellulose and culture medium.

Specification	Glucose	Fructose	Erythrol	Inulin	Lactose
Synthesis efficiency	r(X,Y) =0.918	r(X,Y) =0.710	r(X,Y) =0.788	r(X,Y) =0.933	r(X,Y) =0.866
Water retention	r(X,Y) =0.529	r(X,Y) =0.193	r(X,Y) =0.130	r(X,Y) =0.779	r(X,Y) =0.922
Humidity	r(X,Y) =0.544	r(X,Y) =0.206	r(X,Y) =0.201	r(X,Y) =0.765	r(X,Y) =0.835
The determined correlation coefficients are significant at $p<0.05$					

On the basis of the analysis of the correlation measurements between selected properties and the culture medium we note that in the case of erythrole and fructose the correlation coefficients for water retention and moisture content are just above zero. This means that there is almost no correlation for these measurements. However, high correlations were found between the composition of the culture medium and the efficiency of cellulose synthesis.

The process of cellulose synthesis can be more or less efficient depending on the nutrient used as confirmed by studies (Nakai et al. 1999). Depending on the sugar source, a positive or negative effect of increasing the amount of sugar in the medium on cellulose production can be observed (Naritomi et al. 1997). The reason for this may be that modification of the substrate can accelerate or slow down the activity of cellulose synthesising enzymes (Tahara et al. 2014).

CONCLUSION

Analysing the results of the study, we can formulate the following conclusions:

- The samples containing fructose were characterised by the highest efficiency of cellulose synthesis - 1449%.
- The lowest synthesis efficiency was observed in samples containing erythrole- 6.8 %
- We can therefore unambiguously state that the composition of the culture medium has a significant effect on the efficiency of cellulose synthesis

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Streszczenie: *Wpływ składu podłoża mikrobiologicznego na wydajność syntezy celulozy bakteryjnej.* Głównym celem było zbadanie wpływu składu podłoża hodowlanego na process syntezy celulozy bateryjnej. Wykorzystano 5 różnych składników pokarmowych, będących źródłem węgla dla mikroorganizmów syntetyzujących celulozę: glukozę, fruktozę, erytrol, inulinę oraz laktozę, dodanych do podłoża w trzech różnych stężeniach (1%, 2.5%, oraz 4.5%). Zauważono, że rodzaj oraz ilość składników pokarmowych zawartych w podłożu hodowlanym znacząco wpłynęła na wydajność procesu syntezy celulozy. Zaobserwowano, że najlepsze wyniki wydajności syntezy polimeru osiągnięto na podłożu zawierajacycm 1% zawartość fruktozy.. Ponadto otrzymane wyniki jednoznacznie potwierdzają, że skład podłoża hodowlanego ma znaczący wpływ retencję wody przez polimer, w procesie jego syntezy na podłożach hodowlanych.

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