Immobilized Biocatalysts for the Synthesis of Exopolysaccharides

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Biocatalytic synthesis of polysaccharides (PSs) is one of the promising and topical areas of the development of modern biotechnology. The variety of useful properties (the ability for gelation, the formation of viscous solutions, high adhesive ability, etc.) helps the PSs to find still newer applications in a plethora of fields. Biocatalysts determine the possible range of renewable raw materials which can be used as substrates for such synthesis, as well as the biochemistry of the process and the rate of molecular transformations. The functioning of biocatalysts can be optimized using the following main approaches of synthetic biology: the use of recombinant biocatalysts, the creation of artificial consortia, the combination of nano- and microbiocatalysts, and their immobilization. New biocatalysts can help expand the variety of the polysaccharides' useful properties.

Keywords: polysaccharides ; renewable biomass ; wastes ; biocatalysts

1. Introduction

Biocatalytic synthesis of polysaccharides (PSs) is one of the promising and topical areas of the development of modern biotechnology [1]. The variety of useful properties (the ability for gelation, the formation of viscous solutions, high adhesive ability, etc.) helps the PSs to find still newer applications in a plethora of fields. These include the medicine, pharmaceutical, food, chemical, textile, and oil and gas industries, as well as the immobilization of cells and enzymes, etc. [2][3]. Many PSs have antitumor, prebiotic, antiviral, anti-inflammatory, antioxidant, immunomodulatory effects, facilitating wound healing and tissue regeneration, eliminating pain syndrome, neutralizing the side effects of medications, and stimulating hematopoiesis [4][5][6]. Currently, PSs of plant origin are actively used in the industry; however, plant-based production is necessarily seasonal and depends on weather conditions. Therefore, the interest in PSs synthesized by biocatalysts (BTCs) in the form of cells of various microorganisms (bacteria, fungi, etc.) taken in a suspended or immobilized state is steadily growing.

Microbial PSs are more diverse in composition and properties than those of plant origin. Moreover, via controlling the BTCs' properties and the conditions of biocatalytic processes of biopolymers' synthesis, it is possible to obtain polymers with the desired features and in the required quantities [7]. The microbial PSs are characterized by the presence of a large number of functional groups (hydroxyl, carboxyl, carbonyl, acetate, etc.), which make it possible to modify such biopolymer molecules in order to give them valuable properties [7][8].

In microorganisms considered BTCs and capable of synthesizing PSs, these biopolymers perform a number of diverse functions. These include, in particular, protective, reserve, nutritional, stabilizing ones; besides, PSs determine the immunological properties and virulence of strains, participate in adhesion processes and are responsible for the formation of biofilms. Among the microbial PSs synthesized by various BTCs, intracellular and extracellular biopolymers usually differ in their localization. Intracellular PSs are accumulated inside of BTCs, whereas extracellular PSs, exopolysaccharides (EPSs), are usually secreted into the medium containing the cell and can be separated from BTCs, while the biopolymers themselves can assume the form of capsules, mucus, layers, etc. The interest in EPSs today is mainly due to their unique properties, including those that can benefit mankind, particularly in using EPSs as prebiotics and immunomodulators. It is toward the development of approaches to the biocatalytic production of EPSs and their derivatives that the attention of many researchers is currently directed [9][10][11][12]. Despite the successes achieved in the field of biotechnology of microbial polysaccharides, the number of them produced by industry is extremely limited, and the problem of finding new cost-effective ways to obtain them is still acute. This is largely due to the low yield and high cost of the resulting products. The main ways to reduce costs include using cheap substrates, increasing yields by creating more productive strains using genetic engineering methods, and optimizing cell culture processes. The rate or degree of conversion of a carbohydrate substrate into a polymer product can be increased by improvement of the specific activity of

enzymes involved in synthesis and regulating the biosynthesis pathways of EPS precursors. Another problem in the process of EPSs biosynthesis is the change in the rheological properties of the medium at the stage of EPSs formation, which creates difficulties during mixing and difficulties for mass transfer processes. Despite numerous studies and the creation of productive strains, optimal ways have not yet been found that would allow the creation of mutant strains that fully meet the requirements of industrial production. In addition, the use of genetically modified microorganisms on an industrial scale always has a number of significant limitations, primarily related to ecology. There is also a need to introduce expensive inducers into nutrient media for the biosynthesis of necessary enzymes in such cells and antibiotics to suppress native microflora. Undoubtedly, the development of new highly productive stable biocatalysts, providing, among other things, fundamentally new materials, will remove a number of restrictions on the use of EPSs.

The range of the most significant PSs obtained microbiologically includes pullulan, dextran, bacterial cellulose (BC), alginate, xanthan, levan, curdlan, succinoglycan, and others. The interest in them is primarily due to the variety of their possible practical applications. BTCs play an important role in EPS synthesis, ensuring the flow of interrelated enzymatic biochemical transformations from the initial substrate to the final product. The range of the substrates that can be successfully used for bioconversion and the characteristics of the products thus obtained depend on the BTCs. The latter also determines the possible biochemical transformations and thus influences the choice of methods and conditions for the synthesis of PSs, as well as the speed of the process and the yield of the target product.

2. Immobilized Biocatalysts for the Synthesis of Exopolysaccharides

There are both prokaryotic cells and eukaryotes that prefer aerobic or anaerobic processes among the biocatalysts synthesizing PSs, and this generally determines the diversity of the biocatalytic processes synthesizing various EPSs. The common trait of all these catalysts in such processes is the increase in the concentration of cells and their transition to a quorum-sensing (QS) state. QS ensures the activation of the synthesis of PSs [13][14] as stabilizing, protective, and reserve substances for highly concentrated microbial populations. Therefore, creating biosystems with a high content of cellular biomass producing PSs and supporting the microbial BTCs in such a concentrated and metabolically active state is one of the nature-like approaches to improving the efficiency of BTCs synthesizing PSs. Cell immobilization can significantly improve the productivity and stability of BTCs (**Figure 1**).

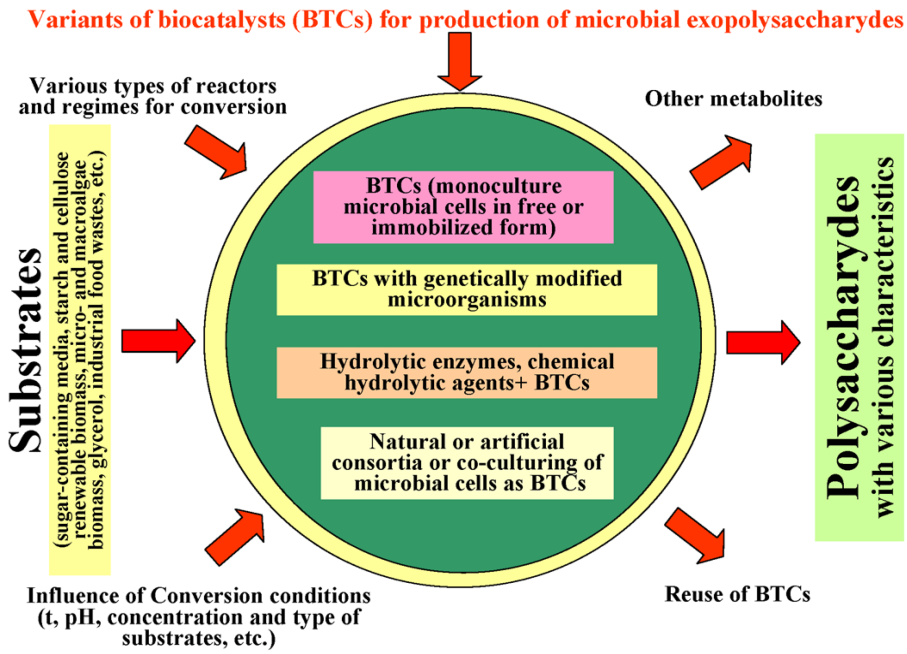

Figure 1. Main variants of BTCs used for producing microbial EPSs from various sources.

It is known that immobilized cells, being in the QS state, can withstand significantly higher concentrations of toxic substances than free cells. Immobilized cells have a higher period of semi-inactivation and can be stored for a long time without a loss of metabolic activity. The immobilization of the cells leads to a change in their genetic and biochemical status, launching various cascade regulatory systems in these cells and the intensification of biochemical processes of basic metabolism. All these factors lead to an increase in the overall productivity, viability, and resistance of these cells [15][16]. This ensures a huge interest in the use of immobilized microbial cells as BTCs for the synthesis of EPSs.

For example, *Lactobacillus rhamnosus* RW-9595M cells immobilized on a solid insoluble carrier (ImmobaSil), which is a silicone polymer, when cultured in a medium containing a serum permeate at a concentration of 5–8 wt.%, were able to

synthesize EPS during 4 working cycles with the accumulation of EPSs in concentration of 1.7 g/L [17]. However, this EPS concentration was lower than that accumulated in the medium with free cells (2.35 g/L), which was due to the problems for mass transfer processes created by the carrier used. At the same time, a high concentration of immobilized cells (8.5×10^{11} cells/g of carrier) led to an increase in EPS productivity (250 mg/L/h), which was almost 2.5 times higher than in the case of free cells (110 mg/L/h).

The continuous process of EPS production, organized using the same immobilized *L. rhamnosus* RW-9595 M cells [18], revealed morphological and physiological changes in the cells, leading to the formation of very large aggregates consisting of cells and EPS themselves, which reduced the level of accumulation of the latter (0.138 g/L). Therefore, the synthesized PS should be removed from the cells to provoke them for further synthesis.

Another study of EPS production in various media with BTCs in the form of immobilized *Lactobacillus delbrueckii* cells *subsp. bulgaricus* using Ca-alginate, k-carrageenan, and a number of other carriers was performed [19]. This research showed that the maximum concentration of EPS can be obtained by culturing cells immobilized in Ca-alginate gel in an Elliker nutrient medium with the addition of sucrose (5 wt.%). The process duration was 18 h at 37 °C and pH 5.5. The productivity of such cells exceeded that of free cells by 46%.

Immobilized BTCs were also used in low-fat cheese production technology, and it was shown that the maximum amount of EPSs (5.7 mg/g of cheese) was formed after 22 days of the process. A study of EPS production using *L. plantarum* MK O2 bacterial cells immobilized in agar and alginate gels [20] showed a EPS yield of 225 mg/L, which was only slightly higher than that in the case of free cells. On the other hand, another study noted an almost fivefold increase in EPS synthesis (0.9 ± 0.1 g/L) by *L. plantarum* MTCC 9510 cells immobilized in Ca-alginate gel when the cells were cultured in a medium containing 40 g/L of lactose for 72 h [21]. Such an increase in the synthesis of EPS by immobilized cells was ascribed to an increase in the cell density per unit volume, as well as to the separation of cells from EPS caused by the carrier.

Until recently, the possibility of BTC immobilization in gel matrices was practically not considered as an acceptable option. It was supposed that such methods could work only in the case of both the substrate and the synthesis product having a low molecular weight [22].

The production of such high-molecular substances as BC by cells immobilized in polymer gels was considered impossible. It was assumed that BC synthesized inside the granules of the gel carrier should block its own secretion into the medium. However, relatively recently, the possibility of efficiently using *Acetobacter xylinum* cells immobilized in Ca-alginate gel for producing a food product based on a BC layer was demonstrated [23]. Moreover, the immobilized cells were found to be capable of being reused in batch mode. The duration of one working cycle was 264 h with an average thickness of the formed BC layer of 0.8 cm. After two working cycles, the viability of the immobilized cells was still high enough. However, Ca-alginate gels are known to have relatively low mechanical strength, which depends on the pH of the reaction medium and the ionic strength of the solutions in which the BTCs are functioning. In some cases, the carrier gels can be destroyed due to cell growth inside the gel matrixes. The metabolic activity of the cells was also found to reduce the operational life of such carriers and, consequently, that of the BTCs [24].

Poly(vinyl alcohol) (PVA) cryogels have high mechanical and thermal resistance, a rigid macroporous structure with variable pore size, and chemical stability in various environments. They have long been successfully used for the immobilization of various microorganisms and their use in environments with a complex chemical composition, under various conditions, pH values and buffering of the medium, and thus can serve as an alternative to alginate gels [25][26][27][28][29][30][31]. Thus, bacterial *Komagataeibacter xylinum* B-12429 cells immobilized in PVA cryogel easily synthesized and “pushed” the formed BC filaments through the pores of the polymer carrier, which eventually merged into a dense gel film without covering the cells.

The latter, thus, were deprived of the possibility of transition to a state of rest, and the synthesis of BC by the cells even became steadily more active [32]. When *K. xylinum* B-12429 cells immobilized in PVA cryogel were cultivated in a medium containing 20 g/L of glucose, the mass of the synthesized BC was 1.6 times higher than that obtained in a suspension culture. Glucose was completely consumed by immobilized cells in 120 h of cultivation. The accumulation of free cells in the medium during the cultivation of the immobilized BTCs was six times less than the concentration of cells in the suspension culture. A more dramatic decrease in pH was also observed in the environment with immobilized cells during the first 70 h of cultivation, which indicated a more intensive formation of metabolites reducing the pH of the medium.

The above-mentioned role of QS in PS synthesis was also confirmed by the following experiment. An increase in the concentration of the producer cells in the composition of immobilized BTCs (from 20 to 40 g dry cell/kg) led to a noticeable

increase in the accumulation of BC in the reaction medium [32]. Note that the specific form of BTCs used in the process (granules or layers) had virtually no effect on the level of BC accumulation. The possibility of reuse of immobilized cells that retain 100% of their metabolic activity for at least 10 working cycles was demonstrated both in media containing pure glucose and those with Jerusalem artichoke hydrolysates. The study of the BC samples synthesized by *K. xylinum* B-12429 cells in free and immobilized form under identical conditions showed that polysaccharide films produced by immobilized BTCs had a greater tensile strength, 30% greater thickness, and a higher degree of polymerization.

Although only a few studies of the use of immobilized cells for PSs biosynthesis have been performed, the high efficiency of such an approach has been proven beyond doubt. The immobilized cells in most cases were in the state of highly concentrated populations and had therefore significantly higher metabolic rates, thus ensuring a higher yield of many target PSs compared to the free cells. The possibility of their long-term functioning (**Table 1**) is yet another advantage of the BTCs' immobilization, allowing an essential increase in the overall efficiency of the BC production. The approach based on the use of immobilized cells makes it possible to obtain PSs from various types of renewable non-food raw materials and biomass, providing an essential advantage over the free cell case in terms of both the process efficiency and the characteristics of the produced PSs (**Table 1**).

Table 1. BTCs based on microbial monocultures for PSs production.

Biocatalyst [Reference]; Features of Cells	Substrate Specificity of BTCs and Main Product (PS)	Conditions for BTCs' Use	Rate of PS Synthesis
	Dextran		
***Leuconostoc mesenteroides* SF3** [33]	**Sucrose-100.0 g/L Dextran-22.5 g/L**	**pH 6.5, 30 °C, 20 h**	**1.13 g/L/h**
***L. mesenteroides* SF3** [34]	**Sucrose-100.0 g/L Dextran-20.8 g/L**	**pH 6.0, 28 °C, 24 h**	**0.87 g/L/h**
***Weissella confusa* R003** [35]	**Sucrose-100.0 g/L/ Dextran-25.0 g/L**	**pH 7.5, 30 °C, 24 h, 125 rpm**	**1.04 g/L/h**
***L. mesenteroides* N7** [36]	**Sucrose 100.0 g/L/ Dextran-13.2 g/L**	**30 °C, 24 h, 100 rpm**	**0.55 g/L/h**
***L. citreum* B-2** [37]	**Sucrose 75.0 g/L/ Dextran-28.3 g/L**	**30 °C, 48 h, 80 rpm**	**0.59 g/L/h**
***L. pseudomesenteroides* XG5** [38]	**Sucrose-125.0 g/L/ Dextran-35.5 g/L**	**30 °C, 48 h**	**0.74 g/L/h**
***L. pseudomesenteroides* G29** [39]	**Sucrose-101.4 g/L/ Dextran-38.4 g/L**	**pH 5.5, 30 °C, 10 h, 200 rpm**	**3.84 g/L/h**
***W. cibaria* 27** [40]	**Sucrose-60.0 g/L Dextran-24.8**	**pH 6.2, 22 °C,24 h**	**1.03 g/L/h**
W. confusa Ck15 [41]	**Sucrose-20.0 g/L Chickpea flour-280.0 g/L Dextran-14.9 g/L**	**pH 6.3, 30 °C, 24 h**	**0.62 g/L/h**
***L. mesenteroides* NRRL B-512F** [42]	**Sucrose-200.0 g/L Milk permeate powder-150.0 g/L Dextran-42.9 g/L**	**30 °C, 24 h**	**1.79 g/L/h**
***L. mesenteroides* MTCC 7337** [43]	**Sugars in sugarcane juice-50.0 g/L Dextran-14.3 g/L**	**pH 7.0, 30 °C, 72 h, 150 rpm**	**0.20 g/L/h**
***L. pseudomesenteroides* DSM20193** [44]	**Sucrose-40.0 g/L Brewers' spent grain-100.0 g/L Dextran-11.1 g/L**	**pH 6.4, 25 °C, 24 h, 200 rpm**	**0.46 g/L/h**
***Lactobacillus mali* CUPV271** [45]	**Sucrose-20 g/L/ Dextran-11.7 g/L**	**pH 5.5; 28 °C; 48 h**	**0.24 g/L/h**
***Weissella cibaria* 10 M; mutant strain** [46]	**Sucrose-171.0 g/L Dextran-14.0 g/L**	**pH 5.4–6.2, 25 °C, 24 h**	**0.58 g/L/h**
***L. mesenteroides* KIBGE-IB22M20; mutant strain** [47] *	**Sucrose-250.0 g/L Dextran-10.5 g/L**	**pH 7.5, 25 °C, 12 h**	**0.88 g/L/h**
***L. mesenteroides subsp. dextranicum* B-5481; immobilized in PVA cryogel** [48]	**Sucrose-200.0 g/L/ Dextran-63.0 g/L**	**pH 7.0, 28 °C, 15 h, 200 rpm**	**4.20 g/L/h; reuse in 5 cycles**

Biocatalyst [Reference]; Features of Cells	Substrate Specificity of BTCs and Main Product (PS)	Conditions for BTCs' Use	Rate of PS Synthesis
L. mesenteroides KIBGE HA1 [49]; immobilized in Ca-alginate gel	Sucrose-100.0 g/L Dextran-8.0 g/L	pH 5.0, 30 °C, 24 h 200 rpm	0.33 g/L/h; reuse in 12 cycles
	Levan		
Bacillus subtilis MTCC 441 [50]	Sucrose-100.0 g/L Levan-30.4 g/L	pH 7.0, 37 °C, 20 h, 150 rpm	1.52 g/L/h
Z. mobilis PTCC 1718 [51]	Sucrose-300.0 g/L Levan-57.0 g/L	28 °C, 48 h	1.19 g/L/h
Bacillus sp. MTCC 1434 [52]	Sucrose-250.0 g/L Levan-61.0 g/L	pH 6.0, 30 °C, 30 h, 100 rpm	2.03 g/L/h
Brachybacterium phenoliresistens KX139300 [53]	Sucrose-300.0 g/L Levan-8.6 g/L	pH 7.8, 30 °C, 72 h, 150 rpm	0.12 g/L/h
B. subtilis (NCIM 5021) [50]	Fresh coconut inflorescence sap (sugars g/L: sucrose–172.3 glucose-16.2, fructose-6.2) Levan-62.1 g/L	pH 6.5, 35 °C, 17 h, 150 rpm	3.65 g/L/h
Zymomonas mobilis CCT4494; immobilized in PVA cryogel [54]	Sucrose-300.0 g/L Levan-81.2 g/L	pH 7.0, 30 °C, 12 h	6.77 g/L/h
Z. mobilis CCT4494; immobilized in Ca-alginate gel [55]	Sucrose-350.0 g/L Levan-21.1 g/L	pH 4.0, 30 °C, 24 h, 200 rpm	0.88 g/L/h; reuse in 12 cycles
Z. mobilis CCT4494; immobilized on sugarcane bagasse [56]	Sucrose-350.0 g/L Levan-32.1 g/L	pH 4.0, 30 °C, 24 h	1.34 g/L/h; reuse in 12 cycles
	Xanthan		
Xanthomonas campestris AM001 [57]	Maltose-70.0 g/L Xanthan-40.7 g/L	pH 7.0, 32 °C, 80 h, 600 rpm	0.51 g/L/h
X. campestris ATCC 13951 [58]	Winery wastewater (sugars g/L-30.7) Xanthan-23.9 g/L	pH 7.0, 29 °C, 96 h, 475 rpm	0.25 g/L/h
X. campestris pv. campestris 1866 and *1867* [59]	Coconut shells or cocoa husks hydrolysates-20.0 g/L (25.0 g/L of sugars) Xanthan-3.6 g/L (coconut shells) Xanthan-4.5 g/L (cocoa husks)	28 °C, 96 h, 250 rpm	0.04–0.05 g/L/h
X. campestris WXLB-006; mutant strain [60]	Glycerol-40.0 g/L (+fed batch 1–3 g/L/h) Xanthan-33.9 g/L	pH 7.0, 30 °C, 60 h, 200 rpm	0.57 g/L/h
X. campestris ATCC 13951; immobilized in polyurethane foam [61]	Sucrose-50.0 g/L/ Xanthan-59.9 g/L	28 °C, 96 h, 180 rpm	0.62 g/L/h; reuse in 12 cycles
X. campestris PTCC 1473; self-immobilized cells on stainless-steel support [62]	Glucose-20.0 g/L/ Xanthan-3.5 g/L	pH-6.9, 30 °C, 47 h, 180 rpm	0.08 g/L/h
X. campestris PTCC1473; immobilized in calcium alginate–polyvinyl alcohol-boric acid gel [63]	Hydrolyzed starch-20.0 g/L Xanthan-9.2 g/L	pH 6.6, 28 °C, 48 h, 180 rpm	0.19 g/L/h; reuse in 3 cycles
X. campestris PTCC 1473; immobilized on plastic (polyethylene) support [64]	Glucose-20.0 g/L Xanthan-8.0 g/L	pH 7.2, 30 °C, 48 h, 180 rpm	0.17 g/L/h
	Alginate		
Pseudomonas stutzeri [65]	Sucrose-20.0 g/L Alginate-5.0 g/L	pH 7.0, 30 °C, 600 h, 200 rpm	0.008 g/L/h
Azotobacter vinelandii 12 [66]	Sucrose-35.0 g/L Alginate-2.7 g/L	pH 7.2, 28 °C, 72 h, 210 rpm	0.04 g/L/h
A. vinelandii, NRRL-14641 [67]	Apple peels-10.0 g/ Alginate-180.6 mg/g	pH 7.5, 38 °C, 48 h	3.76 mg/g/h.
A. vinelandii AT9; mutant strain [68]	Sucrose-20.0 g/L Alginate-3.8 g/L	pH 7.2, 29 °C, 72 h, 200 rpm	0.05 g/L/h

Biocatalyst [Reference]; Features of Cells	Substrate Specificity of BTCs and Main Product (PS)	Conditions for BTCs' Use	Rate of PS Synthesis
A. vinelandii B10436; immobilized in PVA cryogel [22]	Sucrose-30.0 g/L Alginate-2.5 g/L	pH 7.0, 29 °C, 72 h, 200 rpm	0.035 g/L/h; reuse in 5cycles
	Pullulan		
Aureobasidium melanogenum TN1-2 [69]	Sucrose-140.0 g/L Pullulan-114.0 g/L	28 °C, 132 h, 250 rpm	0.86 g/L/h
Rhodosporidium paludigenum PUPY-06 [70]	Sucrose-50.0 g/L Pullulan-21.0 g/L	pH 6.0, 25 °C, 168 h, 150 rpm	0.125 g/L/h
A. melanogenum A4 [71]	Maltose-303.0 g/L/ Pullulan-122.3 g/L	pH 7.0, 30 °C, 120 h, 180 rpm	1.02 g/L/h
A. pullulans Y-4137; immobilized in PVA cryogel [15]	Hydrolysate of Jerusalem artichoke tubers, hydrolysate of potato pulp, hydrolysate of *Chlorella vulgaris* biomass (glucose-15.0–25.0 g/L) Pullulan-3.5–16.8 g/L	pH 5.5, 26 °C, 50 h, 200 rpm	0.07–0.33 g/L/h reuse in 15 cycles
	Bacterial cellulose (BC)		
Lactobacillus hilgardii IITRKH159 [72]	Fructose-50.0 g/L BC-7.2 g/L	28 °C, 384 h	0.02 g/L/h
Komagataeibacter maltaceti [73]	Dextrin-8.0 g/L BC-6.5 g/L	pH 6.0, 30 °C, 134 h	0.05 g/L/h
K. nataicola [73]	Maltose-10.0 g/L BC-5.4 g/L	pH 6.0, 30 °C, 134 h	0.04 g/L/h
K. rhaeticus [74]	Acerola waste hydrolysate-50.0 g/L + glucose 20.0 g/L BC-2.3 g/L	pH 3.6, 30 °C, 288 h	0.008 g/L/h
Medusomyces gisevii Sa-12 [75]	Miscanthus biomass hydrolysate (sugars-20.1–21.2 g/L) BC-1.24 g/L	pH 4.0–4.6, 27 °C, 288 h	0.004 g/L/h
Gluconacetobacter xylinus CGMCC 2955; mutant strain [76]	Glucose-25.0 g/L BC-4.3 g/L	pH 6.0, 30 °C, 360 h	0.012 g/L/h
	Glycerol-20.0 g/L BC-2.8 g/L		0.017 g/L/h
	Aspen sawdust hydrolysate (sugars-42.0 g/L) BC-2.9 g/L		0.017 g/L/h
	Wheat straw hydrolysate (sugars-38.0 g/L) BC-3.6 g/L		0.021 g/L/h
	Rice straw hydrolysate (sugars-40.0 g/L) BC-3.5 g/L		0.021 g/L/h
K. xylinum; immobilized in PVA cryogel [32]	Jerusalem artichoke tubers hydrolysate (sugars-53.0 g/L) BC-4.5 g/L	pH 7.0, 28 °C, 168 h	0.027 g/L/h
	Chlorella vulgaris C1 biomass hydrolysate (sugars-45.1 g/L) BC-2.6 g/L		0.015 g/L/h
	Laminaria saccharina biomass hydrolysate (sugars-36.6 g/L) BC-0.07 g/L		0.0004 g/L/h
	Acanthophora muscoide biomass hydrolysate (sugars-56.0 g/L BC-0.4 g/L		0.002 g/L/h
	Ulva lactuca biomass hydrolysate (sugars-24.1 g/L) BC-0.08 g/L		0.0005 g/L/h reuse in 10 cycles

Biocatalyst [Reference]; Features of Cells	Substrate Specificity of BTCs and Main Product (PS)	Conditions for BTCs' Use	Rate of PS Synthesis
	Fructo-oligosaccharides		
A. pullulans **FRR 5284** [77]	**Sucrose-500.0 g/L Fructo-oligosaccharides-306.3 g/L**	**pH 5.5, 55 °C, 3 h**	**102.1 g/L/h**
A. pullulans **CCY 27-1-94; immobilized on reticulated polyurethane foam** [78]	**Sucrose-200.0 g/L Fructo-oligosaccharides-108.2 g/L**	**pH 5.5, 28 °C, 25 h, 150 rpm**	**4.33 g/L/h**
A. pullulans **CCY 27-1-94; immobilized on walnut shell** [78]	**Sucrose-200.0 g/L Fructo-oligosaccharides-126.5 g/L**	**pH 5.5; 28 °C; 36 h, 150 rpm**	**3.51 g/L/h**
	Curdlan		
Bacillus cereus **PR3** [79]	**Starch-100.0 g/L Curdlan-20.9 g/L**	**30 °C, 96 h, 200 rpm**	**0.22 g/L/h**
Paenibacillus sp. **NBR-10** [80]	**Sucrose-50.0 g/L Curdlan-4.8 g/L**	**pH 7.0, 35 °C, 84 h, 200 rpm**	**0.06 g/L/h**
Agrobacterium sp. **ATCC 13140** [81]	**Juice of discarded asparagus–100 g/L (Sucrose-50.0 g/L) Curdlan-40.2 g/L**	**pH 5.5, 30 °C, 168 h, 200 rpm**	**0.24 g/L/h**
Agrobacterium sp. **DH-2** [82]	**Cassava starch hydrolysate (sugars-90 g/L) Curdlan-28.4 g/L**	**pH 5.5, 30 °C, 96 h, 250 rpm**	**0.30 g/L/h**
Agrobacterium sp. **CGMCC 11546; mutant strain** [83]	**Sucrose-60.0 g/L Curdlan-48.0 g/L**	**pH 5.0, 30 °C, 96 h, 280 rpm**	**0.50 g/L/h**
Agrobacterium sp. **IFO 13140; immobilized on loofa sponge** [84]**;**	**Glucose-50.0 g/L Curdlan-17.8 g/L**	**pH 6.5, 30 °C, 240 h, 150 rpm**	**0.07 g/L/h; reuse in 5 cycles**
	Succinoglycan		
Rhizobium radiobacter **ATCC4720** [85]	**Rice husk hydrolysate-100.0 g/L Succinoglycan-69.0 g/L**	**pH 7.0, 30 °C, 72 h, 100 rpm**	**0.96 g/L/h**
R. radiobacter **ATCC4720** [86]	**Deproteinized whey–50.0 g/L Succinoglycan-13.7 g/L**	**pH 7.0, 30 °C, 192 h,180 rpm**	**0.07 g/L/h**
R. radiobacter **18052 N-11; mutant strain** [87]	**Sucrose-70.0 g/L Succinoglycan-32.5 g/L**	**pH 7.2, 30 °C, 72 h, 250 rpm**	**0.45 g/L/h**
Agrobacterium radiobacter **NBRC 12665; immobilized on loofa sponge** [88]	**Sugarcane molasses-75.0 g/L Succinoglycan-14.1 g/L**	**pH 7.0, 30 °C, 192 h, 180 rpm**	**0.07 g/L/h; reuse in 5 cycles**

* Parameter was estimated by the researchers of the review based on the data in the corresponding publications or taken from the references.

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