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Review Article

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Fermentation parameters and condition affecting Levan production and its applications

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ABSTRACT

Levan an exopolymer is used for drug delivery in medicinal applications. It is a water soluble biopolymer produced by microbial cells both under anaerobic and aerobic conditions which have stronger adhesive properties. This review focuses on the biosynthesis of levan, factors influencing levan production and its applications.

Keywords: Levan, Production and Applications.

INTRODUCTION

Polysaccharides are either produced from plants, algae or from microorganisms such as bacteria, yeast and fungi. In recent years, microbial polysaccharides have wide variety of applications in food, cosmetics, medicine, pharmaceuticals and chemical industries. Levan, a microbial polysaccharide commonly referred as polyfructose, is composed of ß-(2, 6)-fructosyl-fructose units linked by glycosidic bonds [1]. It was produced from sugar juice as an undesirable byproduct which was first proposed by Lippmann, 1881. A strain of *Bacillus sp* was used to produce levan from sucrose [2]. The production of levan will be in plenty upon usage of sucrose-rich substrates catalyzed by the enzyme levansucrase [3]. Levan is non-toxic, non-transparent suspension and reflect visible light [4]. Many types of microorganisms like *Bacillus subtilis, Bacillus polymyxa, Acetobacterlevanicum, Pseudomonas fluorescens,* and *Aspergillus versicolar*are capable of producing levan [5]. Levan is used as emulsifier, stabilizer and thickener.

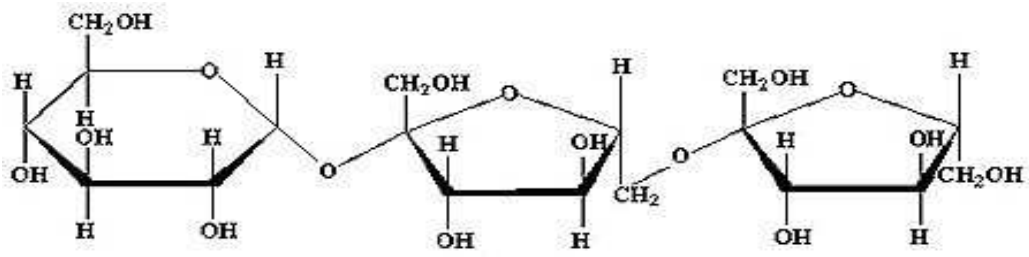

Fig 1: Structure of levan

Biosynthetic pathway of levan

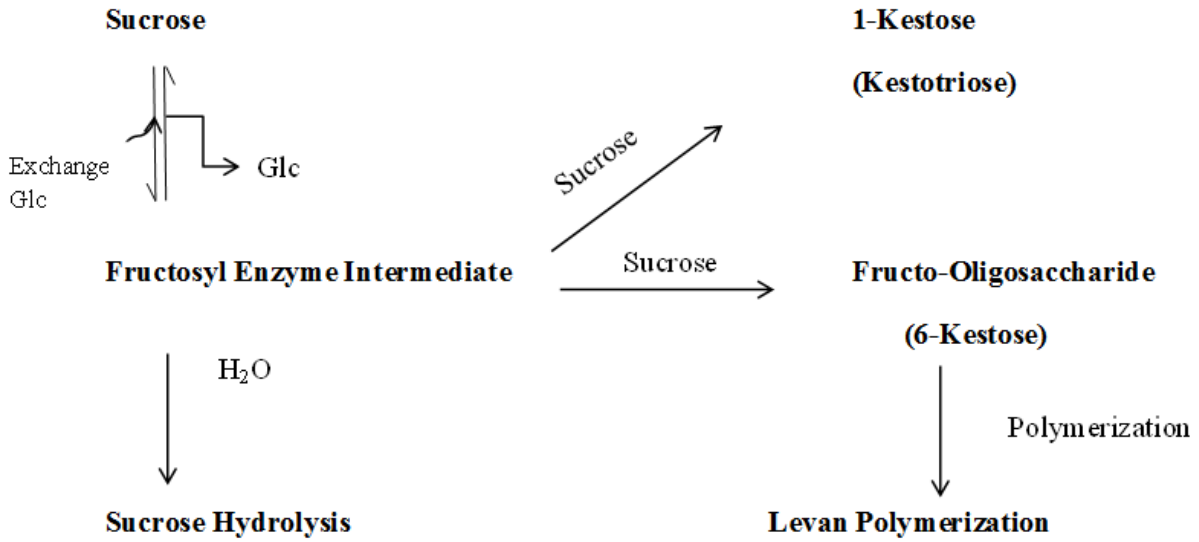

Fig.2: Biosynthesis of Levan

Levan synthesis requires levansucrase, an extracellular enzyme, which increases the productivity of levan when sucrose is provided as a substrate [2]. The levansucrase enzyme catabolizes the sucrose and converts fructose into levan. This reaction is termed as "transfructosylation" [6]. Levan production can be inhibited by sugars like glucose, lactose, D-xylose, L-arabinose, maltose [2].

Properties

High molecular weight levan was soluble in both water and oil. Compared to cold water levan was completely soluble in hot water [7]. Levan was insoluble in most of the organic solvents except Dimethyl Sulfoxide [8]. It has low intrinsic viscosity. Studies reported that, viscosity of levan in water from 0.07-0.18 dL/g and also in DMSO the viscosity range was between 0.20-0.29 dL/g [9]. In a study proposed that, levan from *Microbacterium levaniformis* has the intrinsic viscosity of 0.38 dL/g at 25°C which was greater than levan from other species [10]. It has good thermo stability with a melting point of 225°C [11].

Production of levan from microbial sources

Fig. 3 explains fermentation and downstream processing of levan recovery from microbial system

Table 1: Summary of levan production

S.No	Microorganism	Substrate	Mode of fermentation	Production (g/Lt)	Ref.
1.	*Bacillus subtilis*	sucrose	Batch	70.6	[3]
2.	*Bacillus lentus*	Sucrose	Batch	36.6	[4]
3.	*Bacillus licheniformis*	sucrose	Batch	26	[5]
4.	*Zymomonas mobilis*	sucrose	Batch	40.2	[6]
5.	*Halomonas* sp.	Sugar beet molasses	_	12.4	[12]
6.	*Microbacterium levaniformis*	Date syrup	_	48.9	[13]
7.	*Paenibacillus polymyxa*	Date syrup	_	24	[14]

Now-a-days usage of low cost substrates cuts down the production cost of polymers. Levan from *Halomonas* was produced using molasses as a substrate instead of sucrose and the yield of levan was 12.4 g/L [12]. Date syrup was used as an alternative source for levan formation (10.48 g/L) using *Microbacterium levaniformis* [13]. However, production of levan (24 g/L) from *Paenibacillus polymyxa* was investigated using date syrup [14]. Sugar cane syrup and molasses were used for levan and the yield was found to be 2.533 g/L from molasses which was lower than that from sugar cane syrup 15.456 g/L [15].

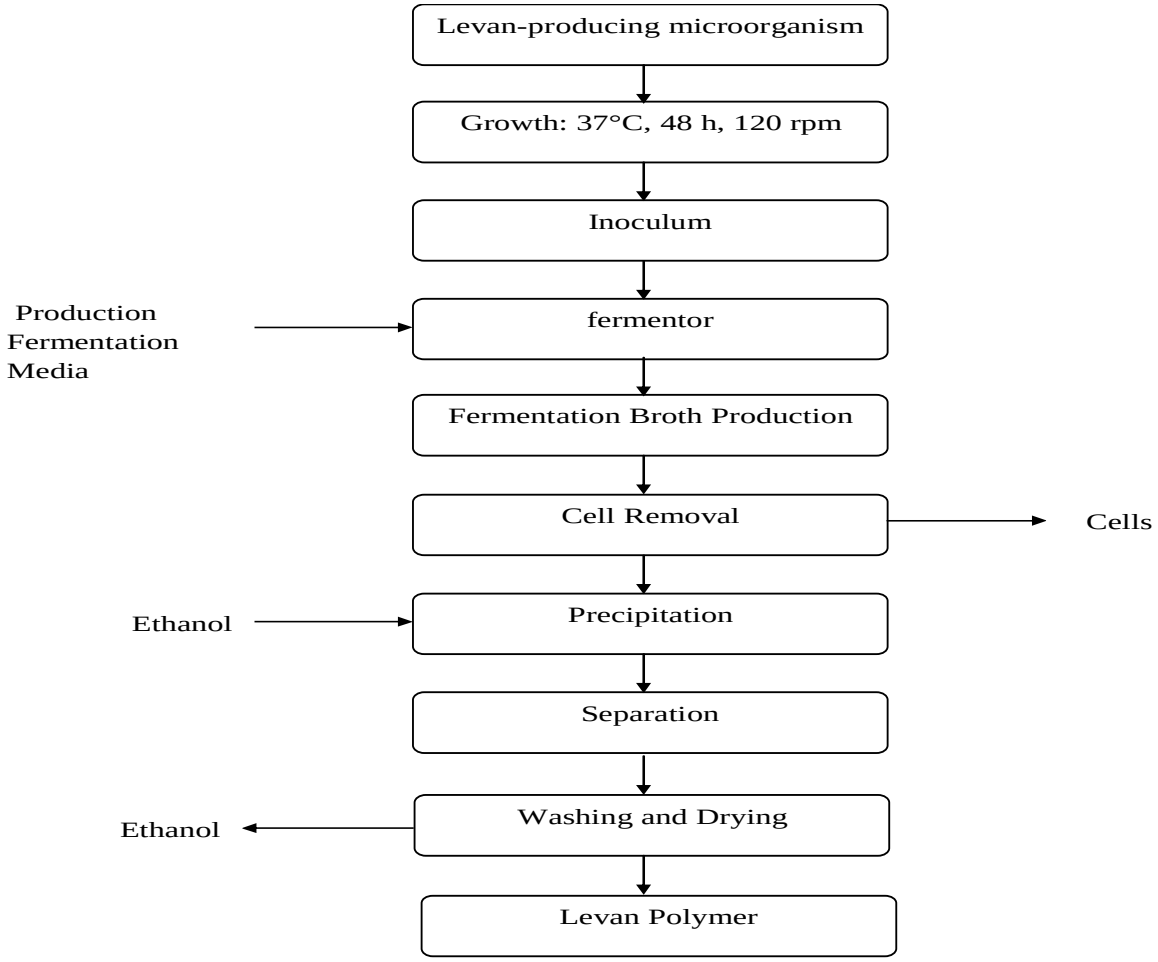

Fig.3: Schematic Representation of Levan Production [13]

Factors influencing levan production

Carbon sources

Carbon sources used for levan production included sucrose, glucose, fructose, galactose, maltose, mannose, raffinose and mannitol [16]. Carbon sources enhance the growth of cell and increases the levan productivity. Sucrose gave the higher yield of levan among most carbon sources. When the sucrose concentration was increased above 300g/L, levan production decreased [17]. Sucrose concentrations from 50 to 200g/L increased the yield of levan from 15.5 to 22.8g/L. But addition of glucose and fructose decreased the production from 27.1g/L to 12.4g/L and 13.1g/L in the case of glucose and fructose respectively [18].

Nitrogen sources and phosphorous salt

Various nitrogen sources used for production of levan were peptone, ammonium chloride, ammonium sulfate and urea [16]. In addition to nitrogen sources, phosphorous salts and metal ions also enhanced the yield of production. There was no significant influence of Ca^{2+}, Na^{+} and K^{+} on levan production but magnesium ion effectively increased the yield of levan up to 15%. It was observed that Fe^{2+} and Zn^{2+} ions retarded the production [17].

Initial pH

Initial pH played a major role in increased cell growth and production. Maximum concentration of levan was produced at pH 6. Negligible levan production was found at pH 4 and decrease in the yield of levan was observed at pH 7. Results showed the formation of levan between pH 5 and 6 [18]. In a study, it was reported that production decreased when the pH values were greater or lesser than pH 6. The optimum pH range for formation was between 5.0 and 6.5 [17].

Temperature

Temperature is one of the significant factors for polymer production. After 24hrs of incubation the highest levan production was obtained at 37°C. Increase in temperature above 47°C reduced levan production. The suitable temperature for levan synthesis was 37°C [17]. The maximum concentration of levan (27.2 g/L) from *Zymomonas mobilis* occurred at 25°C. Negligible concentration of levan was obtained between the temperatures 35°C and 40°C. Yield of levan was high at low temperatures. It was reported that maximum amount of levan concentration was achieved at 7°C [18].

The schematic flow sheet of levan production is shown in fig.

Applications

Studies reported that levan was used in the fields of food, medicine, pharmaceutical and chemical industries. In the area of medicine, levan can be used as an immune modulator, a blood plasma substitute, prolongator, anti- tumor agent [6] and anti hyperlipedemic agent [17]. Levan was applied as both capping agent and reducing agent for synthesis of silver and gold nanoparticles [19]. Levan derivatives can be used in the treatment of AIDS and also as an inhibitor for muscle proliferation. A variety of diseases, caused by fishes due to certain stress factors, can be prevented by Immuno-stimulating ability of levan [20]. It can be used as cell-proliferating and skin moisturizing agents. It does not cause skin irritation [18]. In food industries, levan was used as a sweetener [2], emulsifying, encapsulating agent and a texture forming compound [6]. Other than these applications levan is used in the form of additive, smoothner, equilibrator, enveloper and aromatic enhancer [3].

CONCLUSION

Distinctive properties of levan increase its requirement in many fields like food, medicine and cosmetics. Increasing demand and production cost of levan triggers the researchers to cut down the cost of levan production by using the low cost agro-wastes as substrates.

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