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Study of levan production by *Zymomonas mobilis* using regional low-cost carbohydrate sources

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Abstract

The use of alternative regional low-cost substrates has become very interesting because in addition to the ease of acquisition it presents a relatively low cost. In this study using statistical instruments, the exopolysaccharide levan production by the *Zymomonas mobilis* microorganism was analyzed varying the carbon source (commercial sucrose, molasses and sugar cane syrups) and the fermentation medium constituents. There was a decrease in levan production in the molasses medium (2.533 g L^{-1}) when compared to the commercial sucrose (21.685 g L^{-1}) and syrup (15.456 g L^{-1}) media. Yeast extract and KH_2PO_4 were significant in the commercial sucrose medium for levan production and in the syrup medium the yeast extract and $MgSO_4$ were significant. Although sugar cane syrup up produced about 28.724% less levan compared to commercial sucrose, biomass production in the syrup was 2.76 times greater than in commercial sucrose (0.857 and 2.366 g L^{-1}) that could justify joint levan and *Z. mobilis* ATCC 31821 biomass production. Studies are needed on the use of alternative substrates and complexes in biotechnology to assess the composition of these media, avoiding unnecessary supplementation with vitamins and minerals salts, when using the formulations of the mediums defined for the complex mediums.

Keywords: *Zymomonas mobilis*; Levan; Molasses sugar cane; Sugar cane syrups

1. Introduction

The levan is exopolysaccharide that have a wide variety of applications. The levan can be used in medicine as a hypo-cholesterol [1], antitumor [2], immune modulator [3], anti-inflammatory [4] and plasma substitute [5] agent. It is used in foods as a source of di-fructofuranoses [6], fructose and fructooligosaccharides [7] and as an emulsifying and encapsulating agent, color and flavor vehicle [8] and fat substitute [9].

The *Z. mobilis* ATCC 31821 bacterium is considered a potential candidate for large scale levan production [10]. It is produced from the catalyzed transfructolization reaction by the levansucrase enzyme (β-2,6 frutan: D-glucose-fructosyltransferase, EC. 2.4.1.10) that uses sucrose as substrate. Levan can be produced as economically valuable sub-products in alcohol distilleries using sucrose as substrate [11].

Sugar cane syrup and sugar cane molasses are complex substrates with a high concentration of sucrose and a great variety of salts that are necessary for metabolite production of industrial interest. The sugar cane molasses is noted for its sugar content and sugars usually contribute 60–65% of the solids in sugarcane molasses. Sucrose usually comprises 65–70% of the total sugars with glucose and fructose contributing the highest proportion of the other sugars in molasses. Other carbohydrates usually comprise 10–16% of the solids with pectin compounds and reaction products present in significant quantities. Non-nitrogenous acids contribute 4–9% of the solids with aconitic acid present in significant quantities. Protein and amino acids usually represent only 1–2% of molasses solids. Minerals contribute most (8–17%) of the other solids in molasses. Sugarcane molasses has high concentrations of calcium (1.0–1.1%), magnesium (0.4–0.5%) potassium (3–4%), chlorine (2–3%), and

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sulfur (0.45–0.60%) but it is low in phosphorus (0.1%). Trace metals such as iron, zinc, copper and magnesium are presented in sugar cane molasses.

The osmolarity of the medium is an essential factor for levan and sorbitol production by *Z. mobilis* ATCC 31821, the sodium and potassium salts lead to an increase in the levan and sorbitol synthesis due to the effect of cell osmoprotection [12]. Sugar cane syrup [13], sugar cane molasses [14], sugar beets [15], wheat extract [16], whey [17] have been used in attempts to reduce production costs and use alternative substrate sources.

The use of statistical instruments, such as factorial experimental planning associated to the surface–response methodology, have been used by various authors in biotechnology [18–21]. Experimental planning permits product and process implementation and optimization, reducing development and production costs, because it promotes decrease in the number of experiments at the development stage, so that a considerable number of factors can be studied at the same time. The interaction among these factors can be assessed and optimum production levels obtained and hence greater precision in the results and production optimization [22]. The response–surface methodology is a set of experiment planning and analysis techniques used in the mathematical modeling of responses that can be applied, for example, in pharmacology [23], food sciences [24] and optimization of fermentative processes [25].

The present study assessed, using statistical methodology, the effects of the carbohydrates sources (commercial sucrose, sugar cane syrups and sugar cane molasses), sugar concentration, fermentation time and the influence of the medium constituents in levan production by *Z. mobilis* ATCC 31821.

2. Materials and methods

2.1. Microorganism and culture conditions

The *Z. mobilis* ATCC 31821 bacteria was maintained in culture medium containing in g L^{-1}: sucrose 20, yeast extract 2.5, KH_2PO_4 1, $(NH_4)_2SO_4$ 1 and $MgSO_4(7H_2O)$ 0.5. Media were autoclaved at 121 °C for 15 min. The cultures were kept at 4 °C and renewed every 3 weeks. The inoculum medium contained sucrose at 100 g L^{-1} and salts described previously. The cell concentration was standardized to 0.2 g L^{-1}. The cell concentration was determined by turbidimetry at 605 nm. Batch fermentations were carried out, in 125 ml Erlenmeyer flasks with 25 ml fermentation culture medium at 25 °C.

2.2. Culture media

Three culture media were used, commercial sucrose, sugar cane syrups and sugar cane molasses. The commercial sucrose medium containing in g L^{-1}: sucrose 150, 250 and 350; yeast 2.5 extract, KH_2PO_4 1, $(NH_4)_2SO_4$ 1 and $MgSO_4(7H_2O)$ 0.5. The sugar cane syrups medium was centrifuged, filtered and autoclaved. Commercial sucrose was added until a total sugar concentration of 250 g L^{-1} was reached and the others constituents of the commercial sucrose medium were added. The sugar cane molasses medium was centrifuged, filtered and autoclaved, sugar cane molasses was diluted to a total sugar concentration of 250 g L^{-1}, added to the constituents of the sucrose medium.

2.3. Analytical methods

After each fermentation, the culture was centrifuged (40,000 × *g* for 15 min) and cell growth determined by turbidimetry at 605 nm relating it to a biomass with a dry matter calibration curve. The reducing sugars were quantified according to Somogyi [26] and Nelson [27] using glucose as standard. The total reducing sugars were quantified by the phenol–sulfuric acid method [28]. The levan produced was precipitated by the addition of ethanol to 75% (v/v) at low temperature (5 ± 1 °C) and quantified in fructose units [29].

2.4. Factorial planning

Three successive factorial planning were used to assess the sugar concentration, fermentation time, carbon sources and the constituents of the medium. The first factorial planning was carried out to optimize the sucrose concentration (independent variable x_1) and fermentation time (independent variable x_2) for levan production, using the complete factorial planning of the 3^2 type with two true replications at the central point (Table 1). In the second factorial planning the effects of medium constituents of the sucrose medium on levan production were assessed. The fermentation time (24 h) and sucrose concentration (250 g L^{-1}) were defined from the results obtained in the first planning. Fractioned factorial statistical planning of IV resolution of the 2^{4-1} type was used (Table 2). In the third factorial planning, the sugar cane molasses and sugar cane syrups carbon sources and con-

Table 1
Factorial 3^2 designed to investigate the effects of the sucrose concentration and fermentation time on levan production

Runs	Coded levels		Responses		Levan (g L^{-1})
	x_1	x_2	Biomass (g L^{-1})	Consumption of TRS (g L^{-1})	
01	−1	−1	0.754	22.547	5.231
02	−1	0	0.891	37.612	12.154
03	−1	+1	1.012	51.324	14.123
04	0	−1	0.654	29.847	9.523
05	0	0	0.724	41.856	18.241
06	0	+1	0.857	49.576	21.685
07	+1	−1	0.366	14.258	3.457
08	+1	0	0.481	21.789	8.231
09	+1	+1	0.558	29.234	11.687
10	0	0	0.785	40.587	17.853
11	0	0	0.745	41.687	17.245

Variables		Real levels		
		−1	0	+1
x_1	Sucrose initial (g/L)	150	250	350
x_2	Culture time (h)	12	18	24

TRS: total reduction sugars.