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Optimizing the Conditions of Dextran Synthesis by the Bacterium *Leuconostoc mesenteroides* Grown in a Molasses-Containing Medium

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Abstract—Maximal dextran production (54–55 g/l) by the bacterium *Leuconostoc mesenteroides* strain V-2317D was observed in molasses-containing media in the presence of 17.5% glucose at pH_{init} 6.75. The beginning of dextran production depended on the amount of inoculate; maximum yield was observed at a shaker rate of 200 rpm. The dextran produced by *L. mesenteroides* grown in the molasses-containing medium was representative of three fractions differing in molecular weight and composition: the high- (~54.5%), medium- (~27.9%), and low-molecular-weight (~2.85%) fractions.

The major glue components that are currently used in manufacturing composite materials are very expensive and highly toxic. For this reason, alternative glue compositions based on ecologically safe biological components have been suggested as bonding agents. A glue based on dextrin, the product of hydrolysis of food starch, which is fairly expensive, is widely used today [1, 2]. However, dextrins can be replaced with dextran, a microbial polysaccharide with similar properties. An inexpensive substrate that can be used for obtaining dextran is molasses—a by-product of the sugar-beet industry.

The goal of this work was to study the effect of culturing conditions on the growth of the bacterium *Leuconostoc mesenteroides* in a molasses-containing medium and on the synthesis of dextran.

MATERIALS AND METHODS

In this study, we used the bacterium *Leuconostoc mesenteroides*, strain VKM V-2317D (SF-4), from the collection of OAO Biokhimik (Saransk), which is commonly used for industrial production of dextran.

L. mesenteroides were grown and maintained in a nutrient medium containing KCl (0.1 g/l), $MgSO_4 \cdot 7H_2O$ (0.1 g/l), Na_2HPO_4 (2.5 g/l), KH_2PO_4 (1.0 g/l), NH_4Cl (0.5 g/l), More's salt (0.01 g/l), *p*-aminobenzoic acid (0.05 g/l), peptone (0.2–0.3 g/l), and granulated sugar (100.0 g/l), which served as a control. In addition, we used a medium containing yeast extract (20.0 g/l), $MgSO_4 \cdot 7H_2O$ (0.1 g/l), NaCl (0.01 g/l), $CaCl_2$ (0.02 g/l), KH_2PO_4 (20 g/l), $MnSO_4$ (0.01 g/l), $FeSO_4$ (0.01 g/l), and granulated sugar (40.0 g/l) [3]; pH was adjusted to 6.9 with 1 N H_3PO_4 or 1 N NaOH. The content of sucrose in granulated sugar was 99.7% and corresponded to GOST 26884-86 and GOST 21-94. Bacteria were cultivated at 24 ± 0.1°C at pH_{init} 7.0 ± 0.1. A culture was also grown in the presence of molasses produced by the Romodanovskii Sugar-Beet Works, which was the major component of the culture medium. Molasses (forage treacle), a by-product of the sugar-beet industry, contained 43–52% of sugar, microelements, and organic acids, whose concentration depended on the soil and climatic conditions of growth and the beet cultivar [4]. In our experiments, we used molasses of one lot, which contained 50% sucrose. To obtain inoculate, the culture was grown in a circular shaker (200 rpm) in 250-ml flasks containing 100 ml of culture medium [3] for 24 h under periodic conditions. Experiments on the study of environmental factors on culture growth and dextran synthesis were performed in 250-ml Erlenmeyer flasks containing 100 ml of medium or in an AK-210 fermenter (working volume, 3 l; SKB BP, Pushchino). In all experimental variants, the amount of inoculate of the 24-h culture was 2% of the volume of the experimental medium. When studying the effect of the amount of inoculate on the yield of dextran, we added 1–4 ml of inoculate. To study the effect of pH on the production of dextran, a culture was grown in a molasses-containing medium in the presence of 17.5% sucrose; pH was varied from 5.0 to 8.0 by addition of 1 N NaOH or 1 N H_2SO_4.

Dextran was determined as described in [5, 6]. Dextran was isolated by double precipitation from the culture medium with 96% ethanol (ratio, 1 : 1) and subsequent drying of the pellet at 60°C in an Ikar infrared

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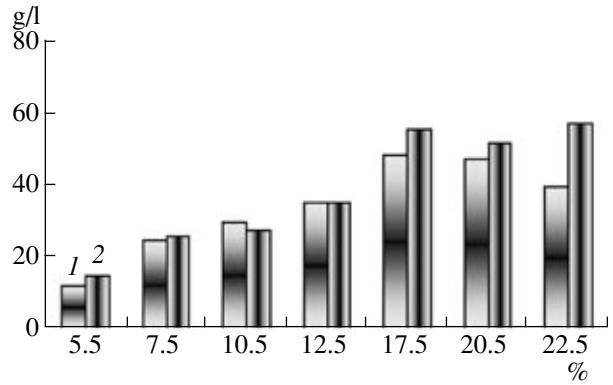

Fig. 1. Dependence of the yield of dextran (g/l) produced by *L. mesenteroides* on the content of sucrose (g/l) in culture medium: (*1*) control sucrose-containing medium and (*2*) molasses-containing medium. Bacteria were cultivated for 4 days in a circular shaker (200 rpm, 24°C, pH_{init} 6.8).

dryer (AO Vologda Opticomechanical Works, Russia). Protein content in cells was determined by the Bradford method.

The activity of dextran saccharase (EC 2.4.1.5, sucrose: 1,6-α-*D*-glucan-6-α-glycosyl transferase) was determined as described in [7, 8]. The amount of the enzyme that produced 1 μmol of reducing saccharides in 1 min was taken as one unit of activity (E) of dextran saccharase. The reaction was performed at 30°C under stirring on a magnet stirrer. The amount of reduced saccharide was determined in aliquots taken at 50 min intervals for 20 min. The concentration of sucrose in molasses was determined polarometrically [6]; the pH of the medium was determined potentiometrically.

RESULTS AND DISCUSSION

Effects of carbon source and pH on the yield of dextran. It is known that the degree of branching of dextran, its viscosity, and its cohesiveness increase in a medium containing molasses but not sucrose [9, 10]. The nature of the source of carbon and its content in the medium determine the yield of dextran. Our experiments showed that, when *L. mesenteroides* B-2317D was cultivated in media containing different quantities of sucrose, the yield of dextran was maximum (49 g/l) on day 4 of culturing in the presence of 17.5% sucrose. Further culturing decreased the yield of dextran (40 g/l on day 5 of culturing). When *L. mesenteroides* was grown in media with a different content of sucrose in molasses, the yield of dextran was also the highest in the presence of 17.5% sucrose (Fig. 1). Further increase in the concentration of sucrose in molasses to 20–22.5% did not increase the yield of dextran. For this reason, all further studies were performed in the presence of 17.5% sucrose in medium irrespective of the nature of the original substrate.

It is known that pH optimum for *L. mesenteroides* cultivated in the medium containing 17.5% sucrose is within the range from 6.5 to 8.0; maximal accumulation of dextran was observed in day 4 of culturing at initial pH 6.25–6.75 [11]. Cultivation of the bacterial culture in the medium containing 17.5% molasses sucrose at pH 5.0 gave a dextran yield of only 6.1 g/l (Fig. 2).

An increase of the initial pH to 6.0–6.5 during cultivation allowed the yield of dextran to be increased six times compared to the culture grown at pH 5.0. The optimal pH for the synthesis of dextran in the molasses-containing medium was 6.75; a further increase in pH to 7.0–8.0 rapidly suppressed dextran production.

A study of changes in pH during cultivation of *L. mesenteroides* in the molasses-containing medium in an AK-210 fermenter with initial pH value of 7.5 showed that the pH decreased to 7.15 after 2 days of culturing (Fig. 3). At this pH value, the dextran content in the culture medium was minimal and reached a maximum (~55 g/l) on only day 5 after ending the exponen-

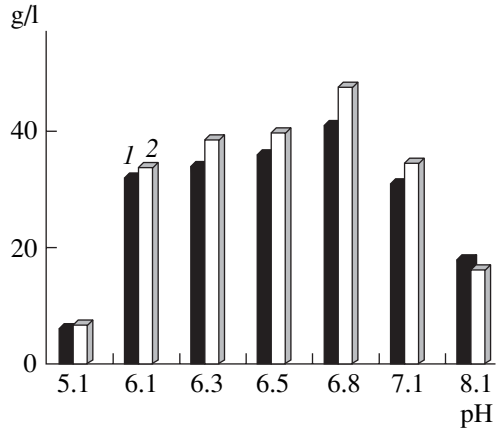

Fig. 2. Dependence of the yield of dextran (g/l) produced by *L. mesenteroides* on initial pH of (*1*) sucrose-containing and (*2*) molasses-containing culture medium. Both variants contained 17.5% sucrose. Bacteria were cultivated for 4 days in a circular shaker (200 rpm, 24°C, pH_{init} 6.8).

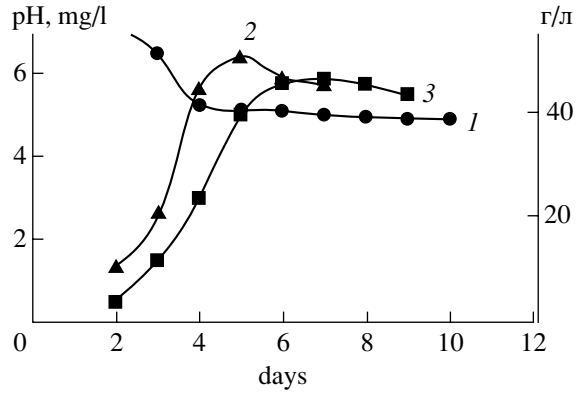

Fig. 3. Time course of (*1*) changes in pH of culture medium, (*2*) dextran synthesis, and (*3*) growth of *L. mesenteroides* cultivated in the molasses-containing medium. Culture was grown for 13 days in an AK-210 fermenter (60 rpm, 24°C, pH_{init} 7.5).

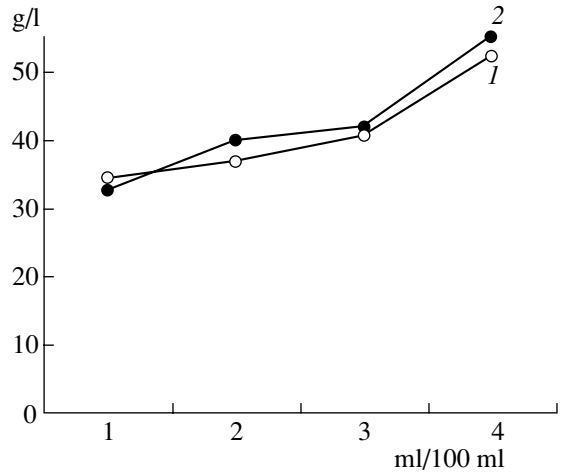

Fig. 4. Effect of the amount of inoculate (ml per 100 ml of medium) on the yield of dextran (g/l) produced by *L. mesenteroides* in (*1*) molasses-containing and (*2*) sucrose-containing medium. Both variants contained 17.5% sucrose. Bacteria were cultivated for 5 days in a circular shaker (200 rpm, 24°C, pH_{init} 6.75).

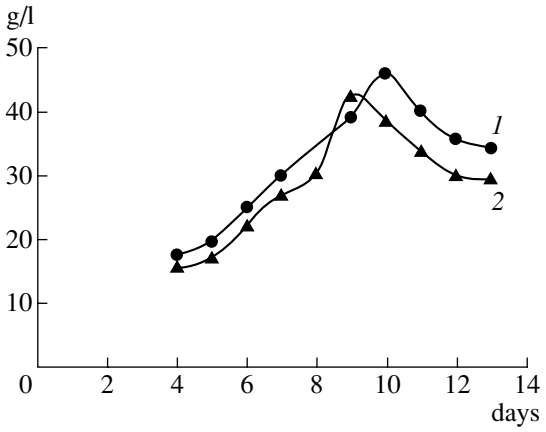

Fig. 5. Synthesis of dextran (g/l) by *L. mesenteroides* grown in an AK-210 fermenter at (*1*) 200 rpm and (*2*) 60 rpm in the molasses-containing medium with 17.5% sucrose without forced aeration (pH_{init} 6.75, 24°C).

tial growth phase at pH 6.4. At pH 5.6–5.8, the amount of biomass reached a maximum on day 6–7 of culturing. At the end of culturing, on day 8–9, the pH decreased to 4.90, which was apparently due to the formation of fermentation products decreasing the acidity of the medium [11].

The rate of dextran production also depended on the amount of inoculate added to the culture medium. When 4 ml (4.52 mg protein/ml) of the inoculate (24 h of culture growth in medium [3]) were added, the yield of dextran reached 54–55 g/l on day 4 of culturing in both the sucrose- and molasses-containing media (which contained the same amount of sucrose) (Fig. 4).

Time course of accumulation of dextran by *L. mesenteroides*. It is known that dextran saccharase is synthesized both under static and dynamic conditions and that aeration with air increases the rate of dextran synthesis [12]. A study of dextran saccharase activity and dextran production by *L. mesenteroides* grown in 500-ml flasks in sucrose-containing medium [3] under different stirring regimes showed that the activity of dextran saccharase was maximal on day 1 of culturing under static conditions (29.3 U/ml). When *L. mesenteroides* were cultured in a circular shaker, the activity of the enzyme was 23.8 U/ml after 2 days of culturing, which was lower by 18% that under static conditions. The activity of the enzyme under stirring of a magnet stirrer (200 rpm) on day 1 of culturing was 19.6 U/ml, which was also lower than under static conditions. Therefore, to isolate dextran saccharase from *L. mesenteroides* strain V-2317D, it is reasonable to cultivate it in this medium under static conditions.

The yield of dextran when *L. mesenteroides* was cultivated in the sucrose-containing medium [3] under stirring on a magnet stirrer (24.9 g/l on day 2 of culturing at 200 rpm) was greater by 19% (20.2 g/l) and 10% (21.9 g/l) than in the case of cultivation in a circular shaker and under static conditions, respectively. In all variants, further cultivation led to a decrease in the yield of dextran, which dropped to 13.1 g/l on day 4. Thus, no direct relationship between the activity of dextran saccharase and the yield of dextran was observed.

Studies of the effect of stirring speed (stirring was performed using an impeller mixer) in the AK-210 fermenter on the yield of dextran (the culture was grown in the molasses-containing medium without forced aeration) showed that the yield was maximal on day 9. Culturing at 200 rpm gave a greater maximal yield (by 3.65 g/l) than culturing at 60 rpm (Fig. 5).

Forced aeration with air (70%) in combination with stirring (60 rpm) only slightly increased the yield of

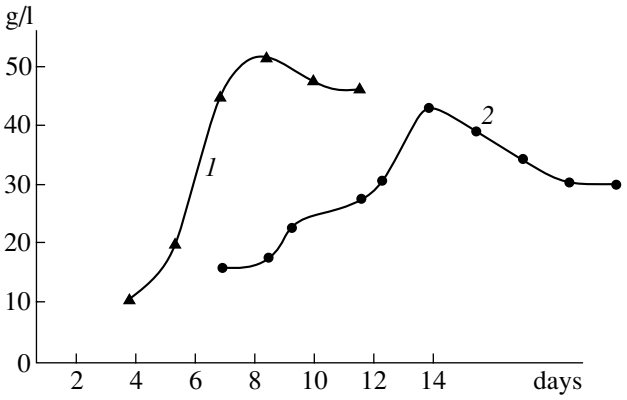

Fig. 6. Effect of forced aeration of culture medium with (*1*) air and (*2*) N_2 on the yield of dextran (g/l) produced by *L. mesenteroides* grown in the molasses-containing medium with 17.5% glucose in an AK-210 fermenter (60 rpm, 24°C).

Fractional composition of dextran synthesized by *L. mesenteroides* grown in a molasses-containing medium, %

Fraction	Sucrose-containing medium (control)	Molasses-containing medium
High-molecular-weight	78.18 ± 0.48	53.6 ± 0.89
Medium-molecular-weight	19.5 ± 0.32	24.83 ± 3.08
Low-molecular-weight	2.5 ± 0.197	2.5 ± 0.35

dextran produced by *L. mesenteroides* in an AK-210 fermenter (Fig. 6). However, it was maximal on day 5, whereas in the case of aeration with N_2 the maximum yield was reached on day 10. This fact is important for industrially obtaining dextran, because a decrease in the duration of its biosynthesis has a significant economizing effect.

Fractional composition of dextran. In the case of industrial production of dextran as a substituent for blood plasma, the conditions that facilitate the synthesis of a linear polysaccharide with a molecular weight of 60–80 kDa, which contains more than 90% of α-1,6-bonds, are ensured. For this purpose, the composition of the culture medium is limited with respect to Mg, which stimulates the synthesis of a branched dextran, and an "inoculum" (20–30-kDa dextran) is added, which ensures the synthesis of dextran of a lower molecular weight [12]. It is known that the molecular weight of dextran synthesized by *L. mesenteroides* varies within the range of 5 to 5000 kDa depending on the culture conditions [2, 8, 12]. Linear fragments of dextran are formed by means of α-1,6-glucoside bonds; branched fragments, α-1,2-, α-1,3-, and α-1,4-glucoside bonds. At the OAO Biokhimik, a medium-molecular-weight fraction (40–70 kDa) of dextran is used in the production of blood substitutes, whereas a high-molecular-weight branched dextran (>1000 kDa) is required to obtain glues.

The dextran obtained in this study, which was synthesized by *L. mesenteroides* culture grown in molasses-containing medium in the presence of 17.5% sucrose, had a dark brown color, which hampered the determination of its molecular weight.

For this reason, the fractions of this dextran were precipitated with different ethanol concentrations. The species that was synthesized by the culture grown in the medium with 17.5% sucrose served as a control. Precipitation of dextran produced by the culture grown in the molasses-containing medium yielded high-, medium-, and low-molecular-weight fractions (53.6, 24.8, and 2.5%; see table). When the culture was grown in the sucrose-containing medium, the content of the high-molecular-weight form of dextran was 23–24% greater than in the molasses-containing medium.

Thus, the results of this study indicate that the maximum yield of dextran produced by *L. mesenteroides* strain VKM V-2317D cultured in the molasses-containing medium is reached under the following conditions: (1) sucrose content in molasses should be at least 17.5%, (2) the bacteria should be cultured in a medium with initial pH 6.25–6.75, and (3) culturing should be performed under stirring. Unlike the data obtained earlier with another *L. mesenteroides* strain [12], to accumulate dextran that predominantly contains the high-molecular-weight fraction, the V-2317D strain should be grown in a sucrose-containing medium.

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