THE SYNTHESIS OF DEXTRAN, I

IMPORTANCE OF SUCROSE CONCENTRATION IN THE COURSE OF DEXTRAN FERMENTATION*

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The industrial production of dextran, considered before as a harmful by-product of beet sugar production, has gained importance since it has been used in big quantities, as e.g. a substitute for blood plasma, as flocculation agent, medicine with iron content or a flotation agent, resp. It can be obtained from sucrose mostly by fermentation, employing the strain Leuconostoc mesenteroides, or by the dextran sucrase enzyme obtained from the microorganism.

The synthesis of dextran is based on the formation of a trisaccharide of G-G-F composition [1] and a fructose from two sucrose molecules. Thereafter some more glucose units are built upon this trisaccharide from other sucrose molecules, accompanied by fructose liberation. The molecular weight of the dextran polymer formed is of the million order. Thus, from the viewpoint of polymer chemistry, sucrose is mainly a glucose donor, but may act as an acceptor at the beginning of a new chain.

Early investigations already proved the importance of sucrose concentration in the molecular weight and the molecular weight distribution of the dextran formed. TSUCHIYA et al. [2, 3] report on the formation of dextran of a mean molecular weight of the million order (entirely precipitated at 38% ethanol concentration) and of about 8000 molecular weight (precipitation begins only over 51% ethanol concentration), for 10% and 70% sucrose concentration, resp., alongside with great oligosaccharide quantities. For a sucrose concentration of 30% and 50%, bimodal molecular-weight distribution was found (one part of the dextran precipitated at 38% ethanol concentration, the other at 46—48% alcohol concentration). Nevertheless, ultracentrifuge tests detected products of low molecular weight in small quantities [3]. The distribution was similar also for different dextran sucrase enzyme concentrations, hence the sucrose concentration seemed to be decisive for the formation of products with different molecular weight distributions [3]. For different original fructose contents (2.5—10%) continuous sucrose dosage changed the molecular weight distribution, too. Thus for 2.5% fructose concentration a

* Dedicated to Prof. Z. CSŰRÖS on the occasion of his 70th birthday.

homogeneous distribution of high molecular weight, at 5% a beginning, and at 10% a definitely bimodal distribution was found. Ultracentrifuge tests showed in the course of the reaction that at 10% fructose content the products of high molecular weight formed mainly in the initial stage of the reaction, while the sedimentation constant of the products with a low molecular weight gradually increased to reaction half-time, thereafter it was constant. Consequently the results of the investigations justify the role of the fructose concentration in the formation of new acceptor molecules, although the molecular weight distribution changes during the reaction, even for constant fructose and sucrose concentrations.

According to Patat and Mayer, at the initial phase of the reaction only high molecular weight products form irrespective of sucrose and fructose concentrations, but towards the end of the reaction also products of low molecular weight appear in significant quantities [4]. Augmentation of sucrose concentration increases the heterodispersity even in the presence of "primary"dextran (controlled synthesis) [5].

For the enzyme synthesis of dextran, the increase of the sucrose concentration reduces the degree of branching of the high molecular weight product [6].

Our investigations covered the experimental observation of the aforesaid enzyme syntheses at the fermentative production of dextran.

Experimental and results

1. *Fermentation experiments:* different sucrose concentrations were applied on 200 ml basic media. Composition of the basic medium:

1% peptone
0.1% KCl
0.55% Na_2HPO_4
0.075% Na_3PO_4
0.33% yeast extract

The basic media, adjusted to different sucrose concentrations, were inoculated with 10% inoculums after sterilization. In all cases 5×200 ml volumes of the basic media of 5, 10, 15, 20 and 50% sucrose concentrations were used. Fermentations were effected in an air thermostat at 28° C.

After different propagation times, a sample of 200 ml was taken from each series. Samples were tested for microbial growth, decrease of pH and sucrose conversion. Molecular weight distribution and molecular structure were determined along with relative viscosity, from the dextrans precipitated at different times by one volume ethanol.

2. *Microbial growth at different sucrose concentrations.* Growth of the Leuconostoc strain was determined by turbidimetry. The values found are not absolute cell numbers; they are characteristic only for the different growth rates measured at different sucrose concentrations (Fig. 1).

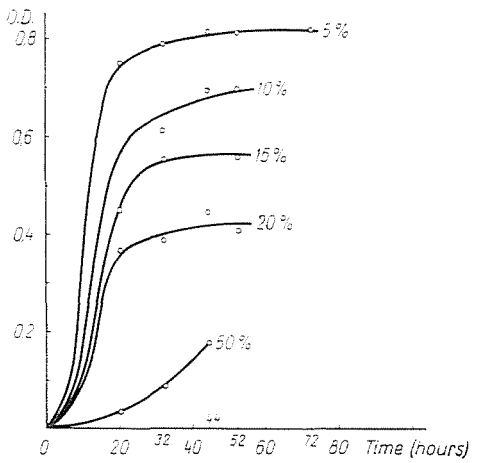

Fig. 1. Growth of microbes for different sucrose concentrations (O. D = optical density in 1 cm cuvettas)

The figure clearly proves that both the growing rate and the possible maximum of relative growth depend mainly on the sucrose concentration, optima being at 5% sucrose concentration.

3. *The rate of dextran formation for different sucrose concentrations.* In dextran fermentation the rate of the synthesis depends on the quantity of the formed dextran sucrase enzyme, on the effect of the organic acids formed in side-reactions upon the hydrogen ion concentration of the medium, on the temperature and on the substrate concentration.

The effect of the sucrose concentration on the rate of the dextran synthesis is shown in Table 1. The effect of the sucrose concentration is more evident from the comparison of dextran formation rates at different times (Fig. 2). Mathematical evaluation of the rate curves, obtained as resultants of a great number of effects is a very complicated task. From the qualitative evaluation of the curves, however, it can be seen that during the first 20 hours, comprehending also the lag period of microbial growth, the rate of dextran synthesis is mediocre. The highest rate was encountered after 32 hours of growth at 5 — 20% sucrose concentrations. At concentrations over 20% always a slower rate was found. For concentrations lower than 20% the rate of dextran synthesis was not found linear to the substrate concentration. The relative rate increase was at its maximum invariably for 5% sucrose concentration. Rate

values at 44 and 72 hours become lower and lower — probably because of the decrease of the pH value of the medium. The role of the pH decrease is proved also by 72 hour dates, showing an increasing rate of synthesis, alongside with the increase of the sucrose concentration, as on account of the 50% concentration and reduced microbial growth the decrease of pH is lower.

Table 1

Quantity of transformed sucrose (g/100 ml)

Fermentation time (hrs)	Sucrose concentration, %			
	5	10	20	50
20	1.06	1.57	2.47	0.75
32	2.76	3.50	6.10	1.60
44	3.90	4.80	8.40	2.08
52	4.25	5.60	—	—
72	5.00	7.20	14.20	4.44
96	—	—	14.40	8.10

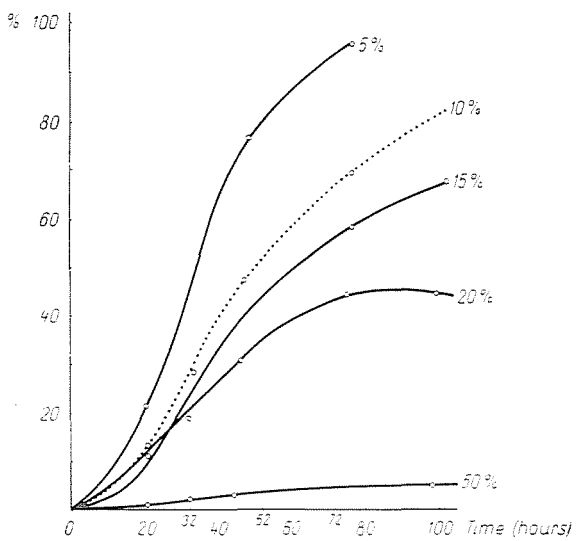

Fig. 2. Sucrose conversion for different sucrose concentrations

4. *Molecular weight, molecular weight distribution and molecular structure at different sucrose concentrations.* There is no exact method to determine the molecular weight of dextran with high molecular weight formed in fermentation. In consequence of the association tendency, light scattering data are generally higher than the real molecular weight, and the intrinsic viscosity data cannot be used practically in this molecular weight range. In fact, the branching degree of dextran changes during the fermentation, thus it cannot be taken into consideration at any molecular weight determination method.

As a consequence, our data are relative viscosity values only, and it should be noted that they are of a qualitative character, and allow to determine any molecular weight or molecular structure only after precise measurement of molecular weight distribution. The relative viscosity of each sample was determined at 20° C and 1% dextran concentration, by means of a modified Ostwald viscosimeter (Table 2).

Table 2

Change in relative viscosity during fermentation

Fermentation time (hrs)	Sucrose concentration %				
	5	10	15	20	50
20	2.90	3.04	2.39	1.49	0.54
32	2.81	2.50	2.32	2.45	1.86
44	2.78	2.47	—	2.65	2.53
52	2.43	—	—	—	—
72	2.50	2.40	2.39	3.93	—
96	—	—	2.34	—	—

Tabulated data show that for 5—10% sucrose concentrations the relative viscosity decreases as a function of the fermentation time, and it increases considerably for 15% concentration. Our observation that the relative viscosity decreases during the growth phase at low sucrose concentrations and at high concentrations increases, may be generalized, too.

The molecular weight distribution of dextran, precipitating by 1 volume ethanol, was determined by turbidimetric titration [7]. As an illustration, Fig. 3 shows the molecular weight distribution of dextrans, obtained by employing 5, 15 and 50% sucrose concentrations in the course of fermentations. It can be seen that at 5% sucrose concentration the quantity of the homogeneous, medium to high molecular weight fraction definitely increases, without changing molecular weight. Some macro-fractions can be found, without any changes in their quantity; and in the low molecular weight fraction cannot be found but some insignificant changes either.

In fermentations at 15% sucrose concentration a definite increase of the molecular weight can be observed. Later, there is no change in the molecular weight, only the ratio of the medium and high fractions increases.

With 50% sucrose concentration a very heterogeneous product distribution was obtained, and the molecular weight definitely increased during fermentation; as molecular weight distribution is concerned, the ratio of higher molecular weight fractions increased at the expense of the products with low molecular weight.

The structures of dextrans obtained with different sucrose concentrations were determined by periodate oxidation. Similarly to the enzymatic

synthesis results of BRASWELL [6], even under fermentation conditions, branching increased as a function of the conversion (thus at 5% sucrose concentration and at 25% conversion 7%, and at complete sucrose transformation 12% other than alpha-1,6 bonds were found in the dextran formed).

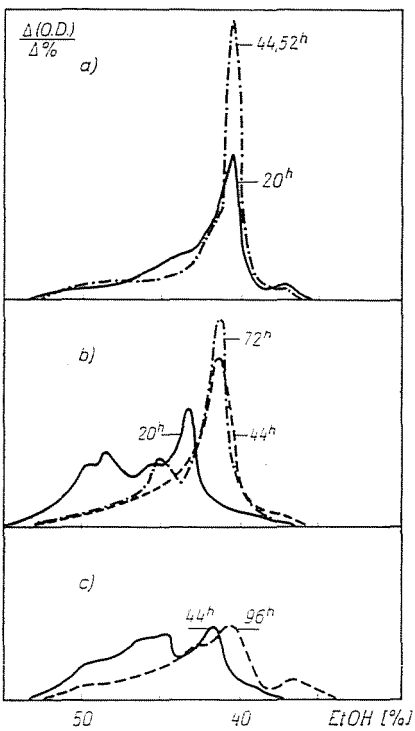

Fig. 3. Molecular weight distribution for 5% (a) 15% (b) and 50% (c) sucrose concentrations

5. *Dextran fermentation under sucrose-stat. conditions.* In conformity with our results obtained at different sucrose concentrations, low sucrose concentrations are advantageous both for the microbial growth and for the relative dextran formation rate, as well as for the molecular weight distribution of the product.

The fermentations were carried out also employing 200 ml volumes. With 5% sucrose concentrations some of the fermentations were stopped and processed, one at a given time, and the sucrose concentrations of the others were adjusted to the sucrose concentration wanted in the next step with 50% sterile sucrose solved in the basic medium.

After treatment and analysis, similar to the foregoing, the results in Table 3 were obtained. It can be seen that the rate of cell growth is high also here during the initial phase and later stoppes, in consequence of rapid pH

decrease. (After the stop, the 5 samples exhibited the following pH values: 5.54; 4.4; 4.51; 4.2 and 4.1).

Repeated sucrose additions allowed to transform greater quantities of sucrose into dextran. In fact, the molecular weight distribution of the dextran formed was more homogeneous than in the single-stage fermentation at a suitable concentration (Fig. 4).

Table 3

Growth of microbes and sucrose conversion

Fermentation time (hrs)	Fictive sucrose concentration (%)	Sucrose conversion (g/100 ml)	Growth*
20	5.0	2.08	0.61
32	7.2	3.10	0.88
44	9.1	4.40	0.90
52	10.9	8.40	0.88
74	12.5	9.60	0.86

* Optical density in 1 cm cuvettas

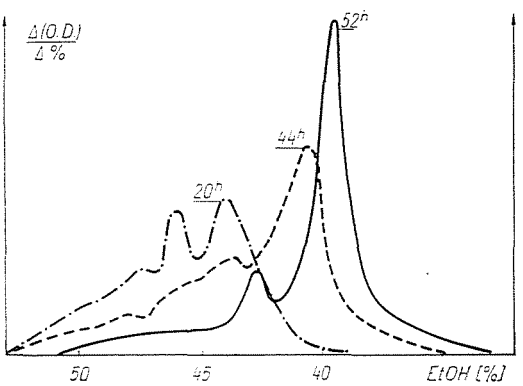

Fig. 4. Molecular weight distribution for constant sucrose concentration

With this method also the branching degree of the product decreases considerably. By the end of the reaction the most linear product was obtained (Table 4).

Table 4

Change in the quantity of alpha-1,6-bonds during fermentation

Fermentation time (hrs)	Alfa-1,6-bonds (%)
20	87.8
32	92.7
44	93.0
52	93.8
74	93.8

Our experimental results lay the foundation for the realization of a semi-continuous dextran fermentation process.

Summary

The effect of sucrose concentration was studied in dextran fermentations with Leuconostoc mesenteroides. The growth rate and the attainable maximum of microbial growth were found to be the highest at 5% sucrose concentration. Accordingly, the relative rate of dextran formation is the greatest at this concentration. During fermentation the relative viscosity of the synthetised dextran decreases at low sucrose concentrations (5—10%), and at higher concentrations (15%) increases. Molecular weight distribution data of the product revealed that at 5% sucrose concentration the quantity of the medium molecular weight fraction changed, but its molecular weight did not. At 15% sucrose concentration the molecular weight increase was found indisputable at the beginning of the fermentation; later, the molecular weight remained constant, only the ratio of the medium fraction increased. At 50% concentration a very heterogeneous distribution of the product was found. Constant sucrose concentration results in optimum dextran formation, the molecular weight distribution of the product is much more homogeneous than in the single-stage system.

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