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Scale-up of Dextransucrase Production by *Leuconostoc mesenteroides* in Fed Batch Fermentation

Georgina L. Michelena*, Aidín Martínez, Antonio Bell, Emilia Carrera and Roxana Valencia
Cuban Institute for Research on Sugar cane By-products; P. O. Box 4026; Havana - Cuba

ABSTRACT

Fed batch fermentation was carried out for the dextransucrase enzyme production from Leuconostoc mesenteroides and the production was scale-up using oxygen transfer criteriuom. It was found that in 5 L vessel fermentation capacity, the best agitation speed was 225 min^{-1} and aeration rate was 0.15 vvm, obtaining dextransucrase activity of 127 DSU/mL.. The maximum enzyme production velocity coincide with the maximum growth velocity between 6 and 7 h of fermentation, which confirmed that dextransucrase production was associated with microbial growth. High enzyme yields were achieved during scale up based on oxygen transfer rate.

Key words: *Leuconostoc mesenteroides*, dextransucrase enzyme, scale-up, oxygen transfer, fed batch fermentation

INTRODUCTION

Dextransucrase is the enzyme responsible dextran production (Kin, 1995;.Haldane, 1994). Its action leads the formation of α (1-6) bonded linear dextran chains and release of fructose in the broth. Traditionally, dextran is produced in a one step process. In the 40's, the synthesis of dextran from cell-free dextransucrase was carried out for the first time (Hehre,1946). Subsequently, different medium compositions and fermentations conditions have been used (Kobayashi, 1986; Lawford, 1979; López, 1980).
Several fermentation methods: batch, fedbatch and continuous culture, as well as the different criteria of aeration have been employed. The immobilization method for production was also studied (El Sayed, 1980).
There is no consesus in literature about the aeration for achieving the best enzymatic activity. Schneider et al. (1984) reported the highest yield under an aeration of 1 vvm where the oxygen level was stayed between 40-80 % of saturation during the fermentation. Monsan et al. (1981) found highest dextransucrase activity in moderate conditions of stirring and aeration, Pennel (1992) and Veljkovic (1992) studied the anaerobic process, Barker (1991) changed aeration from 0.17 to 4 vvm in a 16 l fermenter, and found that high aeration and agitation tended to affect the final enzyme activity. Landon (1990) studied the effect of stirring, suggesting some limitations in difussion through cell membrane in static culture.
The oxygen volumetric mass transfer coefficient, k_{La}, is one of the most important scale up factors in fermentations, and many measuring methods of K_{La} have been proposed (Drapeau, 1986; Vardar-Sukan, 1985; Ju, 1991; Imai, 1987; Rols, 1990). The aim of this study was to optimize the effect of agitation speed and aeration rate on dextransucrase production and to scale up it based on oxygen transfer rate.

* Author for correspondence

MATERIALS AND METHODS

***Dextransucrase production**:* Dextransucrase was produced from *Leuconostoc mesenteroides* B/110-1-1 strain in 3 L of medium in a fermenter of 5 L with the following medium composition: sucrose 2 %, yeast extract 1.5 % and Na_2HPO_4 2%. Fermentation was carried out at 28 °C and pH 6.7 for 7 h. From this point up to hour 9 a sucrose solution of 40 % to constant flux was fed.

***Oxygen transfer conditions**:* It was set up according to the following experimental design:

	Low	Medi	High
S: Agitation speed, rpm	150	300	450
G: Aeration rate, vvm	0	0.15	0.3

Dextransucrase activity was measured as a response variable of agitation speed and aeration rate.

***Dextransucrase assay**:* Dextransucrase activity was expressed in dextransucrase units (DSU). One DSU was defined as the amount of enzyme that converted one miligram of sucrose to dextran in one hour at pH 5.2 and 30 °C in a 10 % sucrose solution. The amount of fructose released was determined using 3,5 dinitro salicic acid method (Miller, 1959).

***Growth measurement**:* Cell growth was measured using a OPTON, PM2A, visible spectrophotometer at 580 nm with 2 ml cuvets. Samples were read against a blank of the initial fermentation broth.

***Dissolved oxygen measurement**:* Measurement of dissolved oxygen was made by means of sterilizable probe (INGOLD, RFA). The probe operated under polarographic principle and consisted of a silver anode and platine cathod separating by a polymeric membrane.

K_{La} determination by gassing out method:
When the fermentation system was in active respiration, aeration was temporaly stopped and the dissolved oxygen concentration (C_L) measurements were made for determinating oxygen uptake rate (QO_2X). Aeration was established and increase in the dissolved oxygen concentration was measured as a function of time. Punctual differential was obtained from gassing curve and C_L vs. $dC_L/dt + QO_2X$ were correlated.

Experimental design and statistical analysis:
The effect of agitation speed and aeration rate on dextransucrase activity were analyzed according to the experimental design and procesed by a computation statistical program.

RESULTS AND DISCUSION

Polinomial models derived from statistical processing of the results obtained in the experimental runs and converted to original variables was as follows:

$$DS\ (DSU/ml) = 107.405 + 0.1011\ S + 71.38\ G - 2.25\ 10^{-4}\ S^2 - 237.71\ G^2 \quad r^2 = 0.933$$

From the model, it can be observed that the increasing in both variables have a value that maximizam the enzimatic activity. It was more damage for the fermentation high level of agitation aereation that work to low level. It seems to be related with the microaereophilic nature of the microorganism.
The difference obtained in sucrose and NaOH uptake (using for pH control) confirmed the influence of the transfer conditions under metabolic activity. Fig. 1 illustrates the effect produced on the biomass due to the differents levels of oxygen transfer.

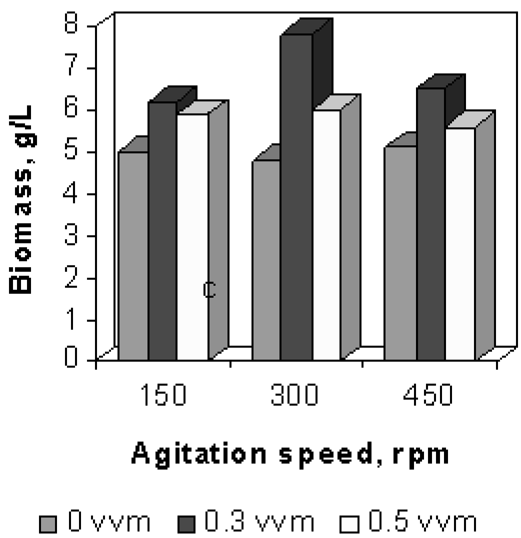

Figure1 - Effect of the differents levels of oxygen transfer on biomass production.

Although the differences are not outstanding, it could be observed that the increase in the aeration levels triggered an strong exponential growing phase of the microorganism, since agitation speed led to a decrease of growth rate after 6 h of fermentation. Lag phase was also affected by experimental conditions, from 3 h in anaerobic fermentation to a significantly lower time as aeration rate increased.

Optimization of the enzimatic activity model yielded a maximum in the following experimentals conditions:

$$\frac{dDSA}{dS} = -4.5 10^{-4} S + 0.101$$

$$\frac{dDSA}{dG} = -475.42G + 71.38$$

S = 225 rpm; G = 0.15 vvm

Fermentation at the optimum condition took place with a maximun of 127 DSU/ml when the time fermentation was 12 h.

Fig. 2 shows the agreement among the maximum of oxygen transfer rate, N_A, enzyme production and growth rate of microbial cells in the range of 6 to 7 h of fermentation. When the regression between growth rate and enzymatic production rate was determined, a lineal correlation was achieved (Veljkovic, 1992). This result seems to be related with the growth associated character of dextransucrase formation from *Leuconostoc mesenteroides.*

During exponential growth phase at this condition, oxygen uptake rate (QO_2X) and K_{La} were determined. Dissolved oxygen uptake QO_2X obtained was 0.0378 g/Lh. A comparison of this value with ones measured with gassing out method for other microorganisms (Kilian, 1983; Smith, 1990) pointed out to be lower than *Candida wickerhamii* and a little higher than *Catharanthus roseus.*

When the air was again supplied, dissolved oxygen as function of time was recorded and K_{La} was determined. Its value was 30.85 h^{-1}.

The rate of oxygen transfer, N_A, during exponential growth was found to be 0.0617 g/Lh, that it was higher than maximun oxygen uptake rate, indicating that the fermentation was not limited by oxygen transfer.

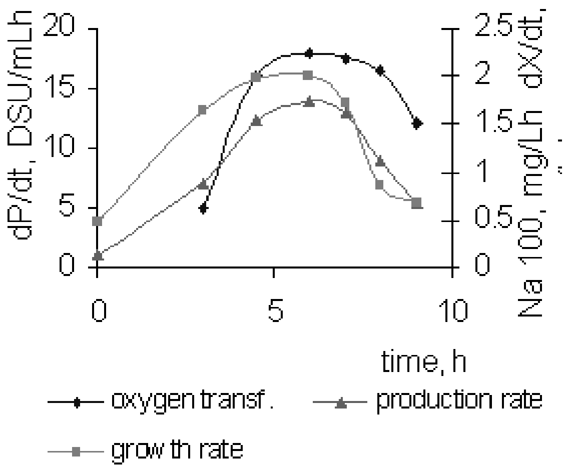

Figure 2 - Behaviour of the oxygen transfer velocity, growth rate and production rate in dextransucrase production.

One procedure of scale up for determining the agitation rate and aeration speed is to fix the power up taken for mixing/unit volumen of liquid, P/V, that is proporcional to fix the k_La.

The aeration number, Na, was calculated for the fermenter of 5 L:

$$Na = \frac{Q}{nD_i^3} = 0.372E - 2$$

With Na and according to the kind of agitator (Aiba, 1970); Pg/P = 0.94 and Re =3.098. 10^4. To the turbulent regimen, Power number, Np = 6 and Power was calculated as:

$$P_1 = \frac{6n^3 D_i^5 \rho}{g_c} = 0.1942 \ \ kgm/s$$

and Pg = 0.186 kgm/s.

As the systems are geometrically similar, from $(Pg/V)_1 = (Pg/V)_2$ and V_2 is known and considering $Np_2 = 6$, it was obtained $n_2 = 90$ rpm.

For calculating aeration speed, Na was kept constant and $Q_2 = 0.016\ m^3$/min and Q/V = 0.05 vvm. With n_2 calculated, Re_2 was checked ($Re_2 = 2.03.\ 10^5$). It was determinated that the regime was turbulent and $Np_2 = 6$ can be used.

In order to confirm the findings, the scale-up was carried out in the 500 L fermenter. Medium sterilization was done for 20 minutes *in situ*, pH was controlled until 7 h an then it was not controlled until pH 5.3. Aeration rate and agitation speed were established according to the values found in 0.05 vvm and 90 rpm, respectively. Dextransucrase activity achieved was 110 DSU/ml, resulting high enzyme yields and short fermentation times at 28 ^oC compared with previous studies (Landon, 1990).

CONCLUSIONS

The influence of agitation speed and aeration rate on dextransucrase production from *Leuconostoc mesenteroides* B/110-1-1 was studied in 5 L vessel. The advantages of microaerophilic system respect to non aerated cultures were demonstrated. Maximum enzyme production coincided with the maximum growth rate and was achieved between 6 to 7 h after the start of fermentation. The scale-up was carried out successfully at 500 L, establishing defined oxygen transfer conditions for improved enzyme production.

RESUMO

Fermentação em batelada alimentada foi realizada visando a produção da enzima dextransucrase a partir de Leuconostoc mesenteroides e o seu escalonamento foi realizado usando critérios da transferência de oxigênio. Foi determinado que em fermentador de vaso de 5L de capacidade, a melhor velocidade de agitação foi de 225 m-1 e a taxa de aeração foi de 0,15 VVM, obtendo uma atividade de dextrasucrase de 127 DSU/mL. A velocidade máxima da produção de enzimaconcide com a velocidade máxima de crescimento , entre 6-7 h de fermentação, o que confirma que a produção dextrasucrase esta associada com o crescimento microbiano. Altos rendimentos na produção da enzima foram obtidos durante o escalonamento baseado-se na taxa de transferência de oxigênio.

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