



highest level of enzyme production is expected in this range [4]. Tsuchiya et al. [4] and Barker et al. [7] showed that when pH drops to a value in the range of 5.0 to 5.5 the enzyme is more active and transforms sucrose to dextran and fructose. Fermentations were carried out by Lazic et al. [8] with controlled pH at pH = 6.7 and 5.5 with similar results. They found that pH = 5.5 and 0.05 vvm of air, are favourable conditions for dextran production and reducing fermentation time.

The strain *L. mesenteroides* NRRL B512(f) is known as micro aerophilic [7,9] and several authors [10–12] consider that oxygen affects positively the strain growth.

*L. mesenteroides* NRRL B512(f) constituent dextransucrase mutants and hyper-producing dextransucrase mutants have now been produced and optimized conditions have been established for the elaboration of the enzyme on a large scale which can allow the production of dextran and fructose in a cell free bioreactor [13,14].

Kinetic studies are a vital part of the overall investigation of product formation in fermentations. A mathematical model allows easy data analysis and provides a strategy for solving problems encountered at the design stage. A fermentation kinetic model can be obtained from published experimental data. The kinetics of some extracellular microbial polysaccharides has been reported [15–17]. These authors developed models for simple reactions where the polysaccharide is produced inside the cell and thereafter expelled to the medium. In our fermentation with *L. mesenteroides*, before dextran production an extracellular enzyme is produced resulting in a more complicated model.

Landon et al. [18] proposed a model of dextransucrase synthesis by *L. mesenteroides*. He considered that cell growth was a linear function of time starting at the end of lag phase. For sucrose consumption only cell metabolism was considered at pH = 6.7 (optimum pH for cell growth). Other authors [1,19] followed dextransucrase production by *L. mesenteroides* and found that this enzyme is a typical growth associated product. Combining all these factors with experimental data it is possible to develop a mathematical model for *L. mesenteroides* fermentation kinetics.

The objectives of this work are:

1. to determine the optimum fermentation conditions for the production of dextran and fructose; temperature, pH, initial sucrose concentration and aeration will be tested.
2. to develop a kinetic model for this fermentation.

## 2. Materials and methods

### 2.1. Strain, inoculum and culture media

The strain used in this work was *L. mesenteroides* NRRL B512(f) obtained from DSM (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH) in freeze-dried vials. The strain was stored at 4°C in MRS agar slants [20] until needed for preparing fermentation inocula. The fer-

mentation inocula were prepared so that the final medium going into the fermenter was 5% (v/v) of the initial fermentation medium. The organism was transferred from the stock culture to a similar medium but without agar (MRS broth). The inoculum was grown for 16 to 17 h at 30°C in agitated flasks.

The fermentation medium contains yeast extract (20 g/dm<sup>3</sup>), K<sub>2</sub>HPO<sub>4</sub> (8 g/dm<sup>3</sup>) and sucrose with variable concentrations. Some authors [2,20] add to the fermentation medium a small quantity of a solution rich in inorganic salts but Landon and Webb [9] found that they were not necessary.

The pH of the medium was adjusted to the value required prior to autoclaving (15 min at 121°C).

### 2.2. Analytical measurements

Bacterial growth was followed by UV spectrophotometry (660 nm) using a Pye Unicam SP6 spectrophotometer. A calibration graph with dry weight versus O.D. (660 nm) gave us biomass concentration in g cells/dm<sup>3</sup>.

Sucrose, fructose and dextran: Substrate consumption and product formation were followed using HPLC equipment. It consists of a Gilson manometric module (model 802 C), a pump (model 302), a refractive index detector and an ion exchange column (Interaction, model 300); connected to a PC.

Dextransucrase activity was assayed by measuring the amount of sucrose consumed. One dextransucrase unit (1DSU) was defined [4] as the amount of enzyme that converts 1 mg of sucrose in 1 h under ideal reaction conditions ( $T=30^{\circ}\text{C}$ ;  $\text{pH}=5.0$ ); in fact 1 DSU = 1/21 U according to the international definition of enzyme activity. Samples were centrifuged to remove cells before analysis. The liquid supernatant (1 ml) was mixed with 1 ml of a 20% (w/v) sucrose solution with pre-fixed pH = 5.0 and then incubated for 1 h at 30°C.

ATP measurements: In a few fermentations ATP (Adenosine triphosphate) was determined using a Microbial Biocounter 2500 (LUMAC, Landgraaf, The Netherlands).

### 2.3. Experimental set-up

In this work, we used a 21 Bioreactor (Setric Genie SET 02, Inchelec, France) with control units for temperature, pH and agitation. The total working volume was 1.5 l. The effect of sucrose concentration, pH, temperature and aeration was studied in batch operation. A peristaltic pump Minipuls 2 (Gilson) was used to collect samples.

## 3. Experimental results

In this work, strain growth and product formation for various operating conditions were studied. Batch fermentations

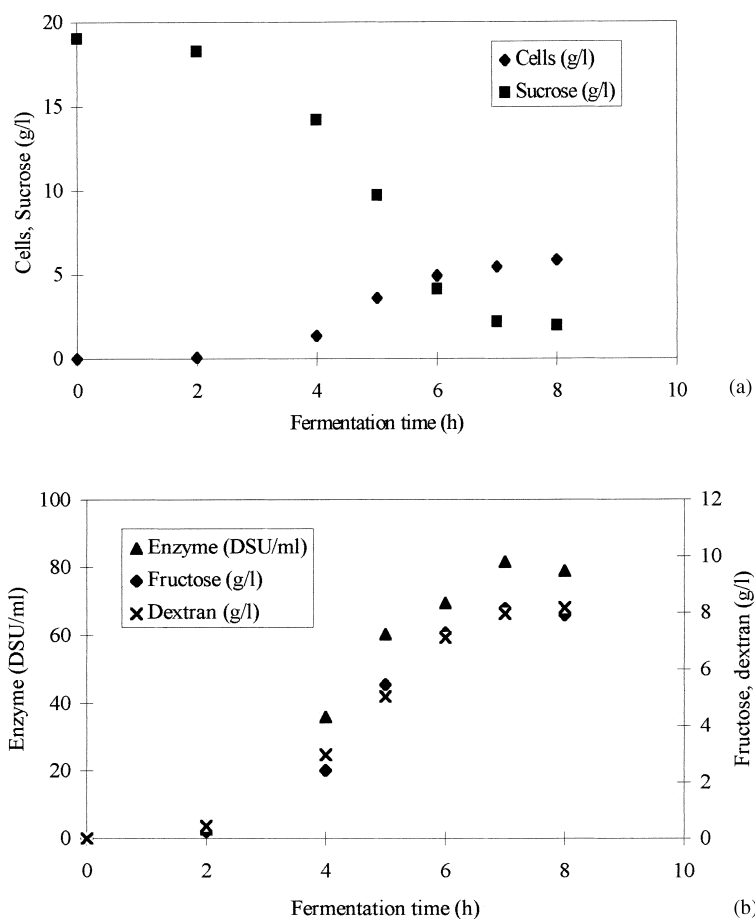


Fig. 1. Batch fermentation run (#31): (a) sucrose consumption and cell growth, (b) enzyme activity and dextran and fructose production. Conditions: pH kept at 5.5 throughout fermentation with 4 M NaOH; temperature = 35°C; Agitation = 150 rpm; no aeration.

Table 1  
Effect of temperature on batch fermentations of sucrose with *L. mesenteroides*<sup>a</sup>

Run	Temperature (°C)	Dextran conc., $D'$ (g/dm <sup>3</sup> )	Fructose conc., $F'$ (g/dm <sup>3</sup> )	Enzyme activity (DSU/cm <sup>3</sup> )	Cell conc., $X$ (g/dm <sup>3</sup> )	Time of fermentation (h)
10	20	6.91	8.16	71.7	5.21	12.0
1	25	6.46	8.09	67.0	5.46	11.5
3	27.5	5.47	7.76	63.2	5.03	11.0
2	30	5.99	7.01	57.2	5.70	10.0
12	35	7.94	8.39	55.6	7.85	7.0
16	40	—	—	11.9	—	15.0

<sup>a</sup> Initial sucrose concentration = 20 g/dm<sup>3</sup>. Without pH control; cell concentration in dry weight of cells/dm<sup>3</sup>.

were carried out for sucrose concentration in the range of 10 to 120 g/dm<sup>3</sup>, temperatures of 20, 25, 27.5, 30, 35 and 40°C, controlled pH of 6.9 (optimum pH for strain growth) and 5.5 (for minimising loss of enzyme activity) and aeration of 0.05 vvm.

Fig. 1 shows an example of a batch fermentation run. Operating temperature was 35°C, pH = 5.5, agitation of 150 rpm, without aeration and sucrose concentration of 20 g/dm<sup>3</sup>. The fermentation took around 8 h and the final concentration of dextran and fructose were respectively 8.17 and 8.15 g/dm<sup>3</sup>. Cellular dry weight reached 5.85 g/dm<sup>3</sup> and enzyme activity reached 81.53 DSU/cm<sup>3</sup>.

### 3.1. Effect of temperature

Generally the temperature used in these fermentations ranged from 23 to 26°C. In this work other temperatures were tested. Table 1 shows results of six fermentations carried out without pH control and no aeration. The cell growth is similar in the range 20 to 30°C, but at 35°C the cell growth is significantly higher and therefore fermentation is faster and takes 4 h less than at 20°C. At 40°C growth does not occur.

The enzyme activity decreases with temperature but dextran and fructose production were not affected; as a matter

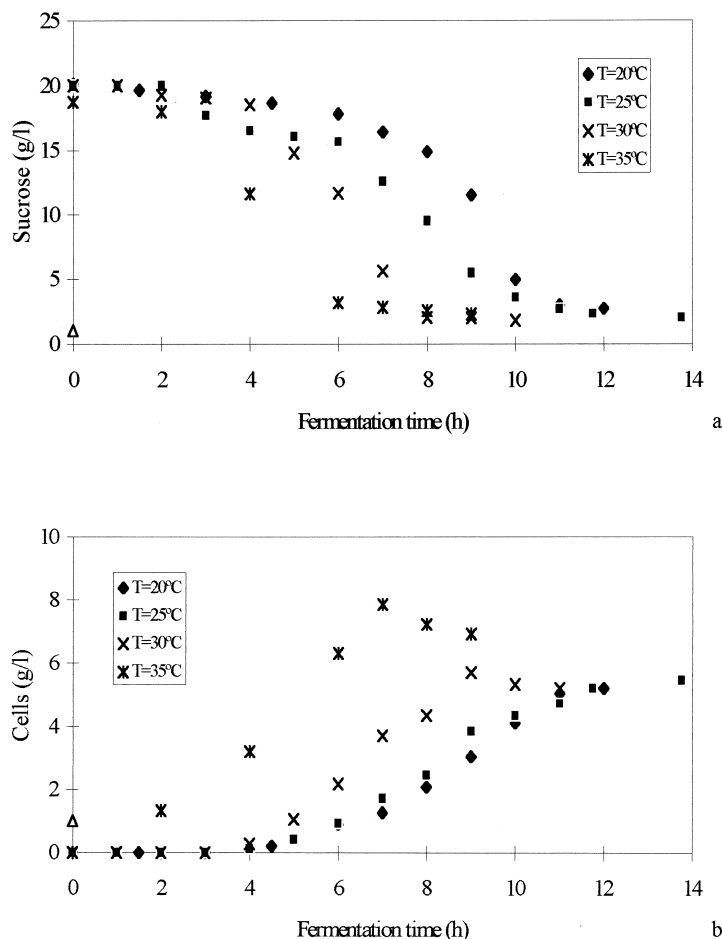


Fig. 2. Effect of temperature (a) on sucrose consumption, (b) on cell growth Conditions: no pH control, Initial sucrose concentration = 20 g/l.

Table 2  
Effect of pH on batch fermentation of sucrose with *L. mesenteroides*<sup>a</sup>

Run	pH	Dextran conc., $D'$ (g/dm <sup>3</sup> )	Fructose conc., $F'$ (g/dm <sup>3</sup> )	Enzyme activity (DSU/cm <sup>3</sup> )	Cell conc., $X$ (g/dm <sup>3</sup> )	Time of fermentation (h)
1	No control	6.46	8.09	67.0	5.46	11.5
4	6.7	6.71	7.01	57.0	7.76	11.0
5	5.5	7.74	8.1	85.0	5.11	8.0
13	5.0	8.51	8.54	51.1	5.11	10.0
11	4.0	—	—	—	0	—

<sup>a</sup> Initial sucrose concentration = 20 g/dm<sup>3</sup>. Temperature = 25°C;  $X$  expressed in dry weight of cells/litre.

of fact higher concentrations of either dextran or fructose are observed at 35°C.

In Fig. 2a and b we compare sucrose consumption and cell growth at different temperatures (no pH control, initial sucrose concentration = 20 g/l).

### 3.2. Effect of pH

The pH 6.7 was taken as the optimum pH for this fermentation by other researchers when they intend to maximize cell growth. Our results are reported in Table 2. It can be seen that indeed pH 6.7 is good for strain growth but at

pH 5.5 the enzyme activity is much higher even with less cell dry weight (Fig. 3a and b). The fermentation time at pH 5.5 is 3 h smaller than for pH 6.7. The results of a standard fermentation (no pH control) are included in Table 2 for comparison. At pH 4.0 growth did not occur and at pH 5.0 growth was similar to that obtained at pH 5.5 but the enzyme activity decreased by 30%.

### 3.3. Effect of aeration

Barker and Ajongwhen [5] demonstrated previously that yeast extract type rather than aeration was much more

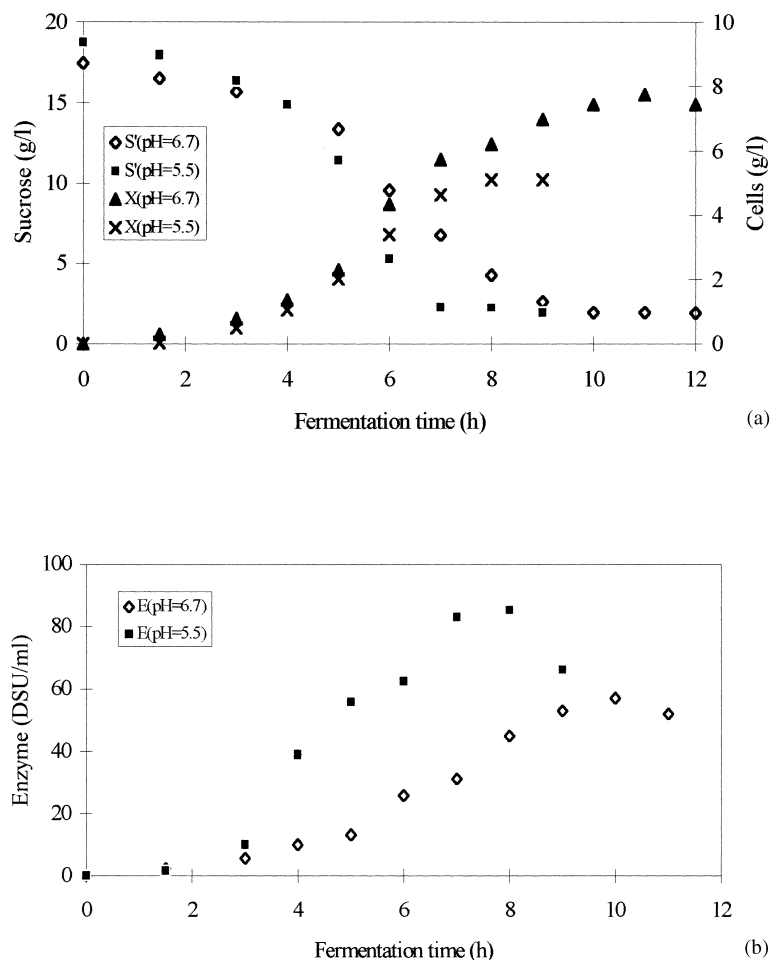


Fig. 3. Influence of pH (a) on sucrose consumption and cell growth, (b) on enzyme production Conditions:  $T = 25^{\circ}\text{C}$ , Initial sucrose concentration = 20 g/l.

Table 3  
Effect of aeration on fermentation of sucrose with *L. mesenteroides* with 0.05 vvm of air at different pH values<sup>a</sup>

Run	Aeration(vvm)	pH	Dextran conc. (g/dm <sup>3</sup> )	Fructose conc. (g/dm <sup>3</sup> )	Enzyme activity (DSU/cm <sup>3</sup> )	Cell conc. (g/dm <sup>3</sup> )	Time of fermentation (h)
5	No aeration	5.5	7.74	8.10	85.04	5.11	8
4	No aeration	6.7	6.71	7.01	57.02	7.76	11
9	0.05	5.5	8.21	8.27	83.42	5.58	9
8	0.05	6.7	7.47	7.60	56.37	7.75	11

<sup>a</sup> Initial sucrose concentration = 20 g/dm<sup>3</sup>. Temperature = 25°C.

important to the results of dextransucrase fermentations but as it was focused in introduction, several other authors [10–12] consider that oxygen affects positively the strain growth.

The average results obtained using the same sucrose concentration, the same yeast extract type and the same agitation and temperature conditions are shown in Table 3. Two pH values were tested: 6.7 and 5.5. It can be seen that aeration does not have an important effect in the fermentation results. It affects positively the strain growth but the enzyme activity decreased with aeration.

### 3.4. Effect of sucrose concentration

Enzyme production only occurs when sucrose is in culture medium. Higher sucrose levels induced better enzyme production but the cultures contained so much dextran that the removal of cells was very difficult. Several runs were carried out with sucrose ranging from 10 to 120 g/dm<sup>3</sup> at pH 6.7 and 5.5. For some runs two temperatures were tested: 25 and 35°C.

Table 4 shows that higher concentrations of sucrose lead to higher dextran, fructose and cell concentration at the end

Table 4  
Effect of sucrose concentration on the production of dextransucrase by *L. mesenteroides*

Run	Sucrose conc.(g/dm <sup>3</sup> )	T (°C)	pH	Dextran conc. (g/dm <sup>3</sup> )	Fructose conc. (g/dm <sup>3</sup> )	Enzyme activity (DSU/cm <sup>3</sup> )	Cell conc. (g/dm <sup>3</sup> )	Time of fermentation (h)
15	10	25	5.5	3.04	2.82	54.01	2.94	7
34	10	35	5.5	3.24	3.28	51.30	3.52	6.5
14	10	25	6.7	2.74	2.69	14.36	4.02	8
5	20	25	5.5	7.74	8.1	85.04	5.11	8
31	20	35	5.5	8.17	8.15	81.53	5.85	7
4	20	25	6.7	6.71	7.01	57.02	7.76	11
32	20	35	6.7	6.89	7.09	50.34	8.02	10
7	40	25	5.5	13.53	15.78	107.29	6.51	9.5
33	40	35	5.5	16.24	18.94	101.92	7.86	9
6	40	25	6.7	11.91	13.77	76.91	9.46	10
19	80	25	5.5	20.06	22.73	62.42	8.74	11
20	80	35	5.5	24.63	24.98	60.02	10.86	9
17	80	25	6.7	19.69	21.79	42.86	13.04	12
18	80	35	6.7	22.9	23.89	41.53	13.50	10.5
22	120	35	5.5	34.56	36.19	89.23	12.42	10
21	120	35	6.7	44.21	46.95	42.94	15.52	11

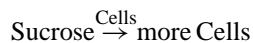
of fermentation. The enzyme activity increases with sucrose concentration up to 40 g/dm<sup>3</sup> of sucrose. For sucrose concentrations of 80 and 120 g/dm<sup>3</sup>, the enzyme activity measurements are difficult because some enzyme and even some dextran and fructose are centrifuged together with cells. A test was made to the centrifuged material by washing it with pure water and centrifuging again. Traces of dextran and fructose were found as well as some enzyme activity. This is difficult to quantify because during the washing process the enzyme loses activity.

Fig. 4a and b show sucrose consumption and cell growth for different initial sucrose concentrations (pH = 5.5 and T = 25°C).

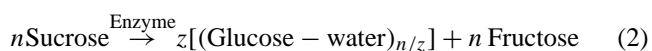
## 4. Kinetic model

### 4.1. Bioreactions and stoichiometry

Sucrose is consumed by *L. mesenteroides* NRRL B512(f) allowing bacteria to grow and to produce the extracellular enzyme dextransucrase. The enzyme uses sucrose in the media to produce dextran and fructose. During the fermentation process the following reactions occur:



Dextran is a polymer of glucose units, so we can write the equation of sucrose consumption by the enzyme in the following way:



where  $n$  is the number of moles of sucrose available in the reaction and  $z$  is the number of moles of dextran produced containing  $n/z$  moles of glucose.

Mass balances should be written for each species.

For cells we have:

$$\text{Cells : } r_X = \frac{dX}{dt} = \mu X \quad (3)$$

where  $r_X$  is cell growth rate (g/l/h),  $X$  is cell mass concentration (g/l),  $t$  is time (h) and  $\mu$  is specific growth rate (h<sup>-1</sup>).

For the enzyme production the following balance holds:

$$\text{Enzyme : } r_E = \frac{dE}{dt} = Y_{E/X} \frac{dX}{dt} \quad (4)$$

where  $r_E$  is enzyme production rate (DSU/ml/h),  $E$  is enzyme produced (DSU/ml), and  $Y_{E/X}$  is the yield coefficient of enzyme production based on the mass of cells produced (DSU/mg cell).

For fructose and dextran molar balances were considered and a first order law both in substrate and enzyme concentrations was assumed, leading to:

$$\text{Fructose : } r_F = \frac{dF}{dt} = nkE(t)S(t) \quad (5)$$

$$\text{Dextran : } r_D = \frac{dD}{dt} = zkE(t)S(t) \quad (6)$$

where  $r_F$  and  $r_D$  are fructose and dextran production rates (mol/l/h),  $F$  and  $D$  are fructose and dextran molar concentrations (mol/l),  $S$  is the sucrose concentration (mol/l),  $k$  is the kinetic constant (ml/DSU h) and  $n$  and  $z$  are the stoichiometric coefficients for fructose and dextran, respectively (see Eq. (2)). By using mass concentrations (g/l) for fructose dextran and sucrose,  $F'$ ,  $D'$  and  $S'$ , respectively, Eqs. (5) and (6) become:

$$r_F = \frac{1}{M_F} \frac{dF'}{dt} = nkE(t) \frac{S'(t)}{M_S} \quad (7)$$

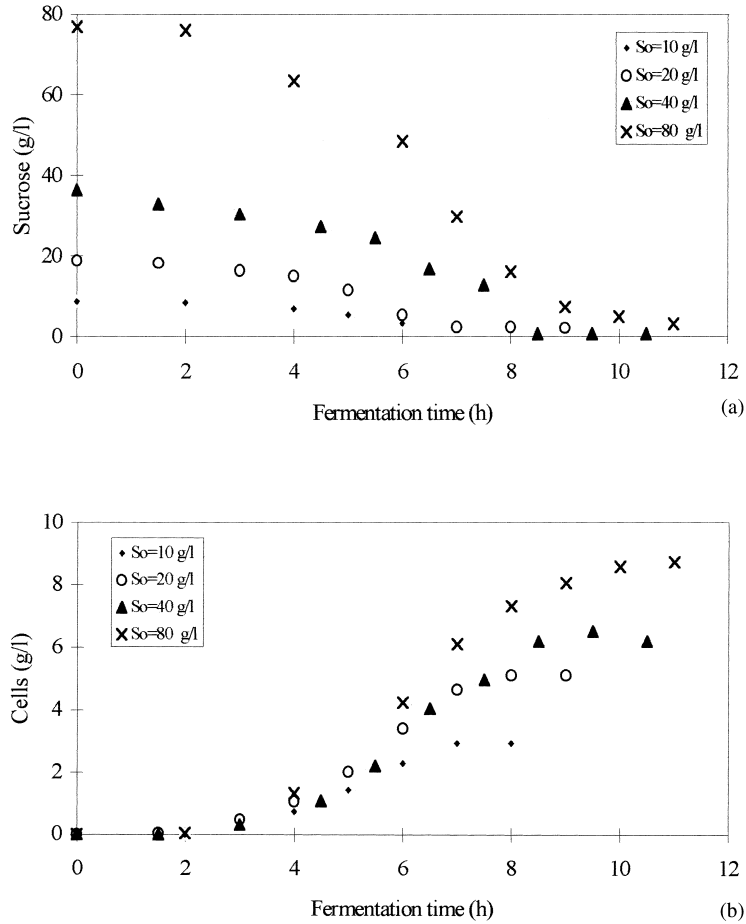


Fig. 4. Effect of initial sucrose concentration (a) on sucrose consumption, (b) on cell growth Conditions: pH=5.5, temperature = 25°C.

$$r_D = \frac{1}{M_D} \frac{dD'}{dt} = zkE(t) \frac{S'(t)}{M_S} \quad (8)$$

where  $M_F$ ,  $M_D$  and  $M_S$  are fructose, dextran and sucrose molar masses (g/mol). From Eq. (2) we are able to relate dextran molar mass  $M_D$  with glucose ( $M_G$ ) and water ( $M_W$ ) molar masses:

$$M_D = \frac{n}{z} (M_G - M_W) \quad (9)$$

The fructose and dextran production rates expressed in g/l/h are then:

$$r'_F = \frac{dF'}{dt} = k' \frac{M_F}{M_S} E(t) S'(t) \quad (10)$$

$$r'_D = \frac{dD'}{dt} = k' \frac{(M_G - M_W)}{M_S} E(t) S'(t) \quad (11)$$

where  $k' = nk$ .

Landon et al. [18] proposed a model of dextransucrase synthesis by *L. mesenteroides* and considered only sucrose consumption by cell metabolism. In the model proposed in this work we consider sucrose consumption by biomass and

also by the extracellular enzyme produced through the cell metabolism, i.e.,

$$\Delta S = \Delta S_{\text{Assimilated into biomass}} + \Delta S_{\text{Used by extracellular enzyme produce dextran and fructose}}$$

For sucrose we have then the following mass balance:

$$\text{Sucrose: } -r'_S = \frac{dS'}{dt} = \frac{1}{Y_{X/S}} \frac{dX}{dt} + nkE(t)S'(t) \quad (12)$$

where  $r'_S$  is sucrose consumption rate (g/l/h), and  $Y_{X/S}$  is the growth yield in the fermentation (g cells/g sucrose).

#### 4.2. Cell growth modelling

A sigmoidal shape is observed in the cell growth curve. The approximation used for cell growth is the logistic equation that characterises cell growth in terms of the maximum value obtained. An approximation usually done [15–17] relates the specific growing rate  $\mu$  with  $X$  as follows:

$$\mu = \mu_m \left( 1 - \frac{X}{X_{\max}} \right) \quad (13)$$

where  $\mu_m$  and  $X_{\max}$  are the maximum specific growth rate and maximum attainable biomass concentration, respectively. The cell growth rate  $r_X$  is then:

$$r_X = \frac{dX}{dt} = \mu X = \mu_m \left(1 - \frac{X}{X_{\max}}\right) X \quad (14)$$

Integrating Eq. (14) with the initial condition  $X(0) = X_0$  (initial cell concentration) we have the logistic equation:

$$X(t) = \frac{X_0 \exp(\mu_m t)}{1 - (X_0/X_{\max})(1 - \exp(\mu_m t))} \quad (15)$$

or

$$\ln\left(\frac{\bar{X}}{1 - \bar{X}}\right) = \mu_m t - \ln\left(\frac{X_{\max}}{X_0} - 1\right) \quad \text{with } \bar{X} = \frac{X(t)}{X_{\max}} \quad (16)$$

As an example: from experimental data (fermentation #31 with  $T = 35^\circ\text{C}$  and  $\text{pH} = 5.5$ )  $X_{\max} = 5.85 \text{ g cells/l}$ , and a plot of  $\ln[\bar{X}/(1 - \bar{X})]$  versus time we get a straight line with a slope  $\mu_m = 1.423 \text{ h}^{-1}$  and an intercept  $X_0/(X_{\max} - X_0) = 0.894 \times 10^{-3}$  corresponding to  $X_0 = 0.00523 \text{ g/l}$ .

Fig. 5a shows the experimental cell concentration  $X(t)$  as a function of time and comparison with model results from Eq. (15).

The parameters  $\mu_m e X_0$  were also calculated for other experiments and reported in Table 5. Parameters  $\mu_m e X_0$  change with initial sucrose concentration, with pH and with operating temperature in each experiment. From Table 5 we can observe that  $\mu_m$  presents higher values for  $35^\circ\text{C}$  and  $\text{pH} = 5.5$ . It also tends to decrease as initial sucrose concentration increases. With respect to  $X_0$  values they are not affected by changes in temperature, pH or initial sucrose concentration. However, the problem is that two inocula are

Table 5

Model equations, input data, calculated parameters for batch fermentation of sucrose to produce dextran and fructose

$$\frac{dX}{dt} = \mu_m \left(1 - \frac{X}{X_{\max}}\right) X$$

$$\frac{dE}{dt} = Y_{E/X} \frac{dX}{dt}$$

$$\frac{dS'}{dt} = -\frac{1}{Y_{X/S}} \frac{dX}{dt} - k' E S'$$

$$\frac{dF'}{dt} = k' \frac{M_F}{M_S} E S'$$

$$\frac{dD'}{dt} = k' \frac{M_G - M_W}{M_S} E S'$$

Initial conditions:

$t = 0; S = S_0; X = X_0$

Input data:  $X_{\max}; E_{\max}; S_0$

Calculated parameters from input data:

$X_0; \mu_m; Y_{E/X}; Y_{X/S}$

The parameter  $k'$  is optimized.

never the same. They are prepared exactly in the same way but the number of viable cells present on the agar slants previously prepared and conserved at  $4^\circ\text{C}$  are never the same. This aspect has a very strong influence on the inoculum final cell concentration originating differences on the calculated  $X_0$  values in the beginning of each fermentation.

#### 4.3. Dextranucrase production modelling

The enzyme concentration as a function of time can be obtained by replacing  $dX/dt$  in Eq. (4); after integration we get:

$$E(t) = Y_{E/X} X_0 \left( \frac{\exp(\mu_m t)}{1 - (X_0/X_{\max})(1 - \exp(\mu_m t))} - 1 \right) \quad (17)$$

The yield  $Y_{E/X}$  was calculated using experimental results from fermentation #31:

$$Y_{E/X} = \frac{E_{\max} - E_0}{X_{\max} - X_0} = \frac{81.53 - 0}{5.85 - 0} = 13.95 \frac{\text{DSU}}{\text{mg cells}} \quad (18)$$

Fig. 5b compares the model results for the enzyme activity  $E(t)$  with experimental data.

#### 4.4. Sucrose consumption, dextran and fructose modelling

##### 4.4.1. The biomass yield $Y_{X/S}$

To solve  $-r'_S$  (Eq. (12)) we need to calculate the biomass yield  $Y_{X/S}$ . This yield was calculated only with relation to the amount of sucrose used for cell growth i.e., the initial sucrose concentration (19.02 g/l) minus the sucrose consumed to produce fructose and dextran minus the residual sucrose. This sucrose quantity can be calculated using the following mass balance:

$$-\frac{dS'_{\text{used by the enzyme}}}{dt} = \frac{M_S}{M_F} \frac{dF'}{dt} = \frac{M_S}{(M_G - M_W)} \frac{dD'}{dt} \quad (19)$$

Integrating Eq. (19) we have:

$$S'_{\text{used by the enzyme}} = \frac{M_S}{M_F} F'_{\text{final}} = \frac{M_S}{(M_G - M_W)} D'_{\text{final}} \quad (20)$$

The final concentration of fructose will be used to calculate the amount of sucrose consumed by the enzyme because these experimental results for fructose are more accurate than those for dextran. The final fructose concentration was 8.15 g/l; from Eq. (20) it can be concluded that the sucrose used by enzyme was  $1.89 \times 8.15 \text{ g/l}$ . Since the final concentration of sucrose was 0.96 g/l, the yield  $Y_{X/S}$  can now be calculated:

$$Y_{X/S} = \frac{5.85}{19.02 - (1.89)8.15 - 0.96} = 3.57 \text{ g cell/g sucrose} \quad (21)$$

We are now able to solve Eqs. (10)–(12) in order to get dextran, fructose and sucrose concentrations as a function of



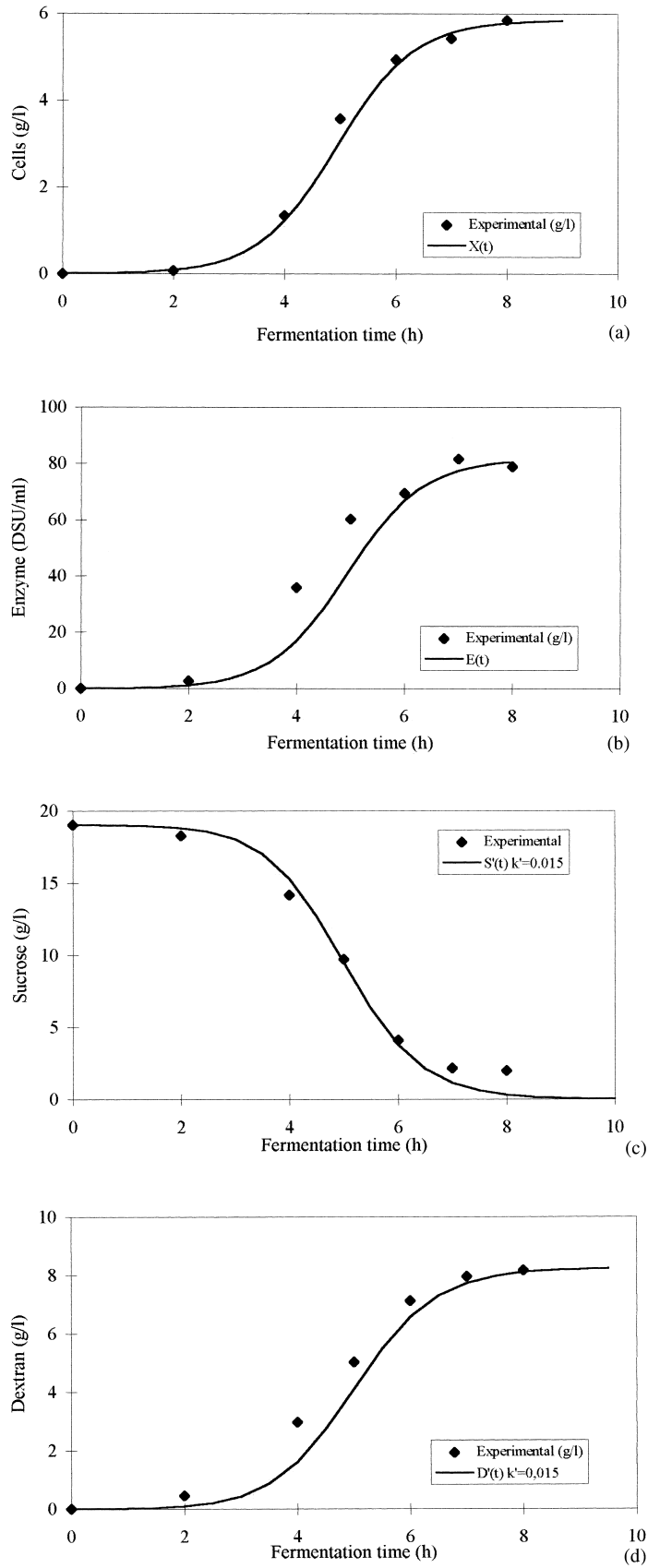


Fig. 5. Model results compared with experimental data from fermentation #31 for: (a) cell growth, (b) enzyme production, (c) sucrose consumption, (d) dextran production and (e) fructose production. Conditions: Initial sucrose concentration = 20 g/l,  $T = 35^\circ\text{C}$  and  $\text{pH} = 5.5$ .

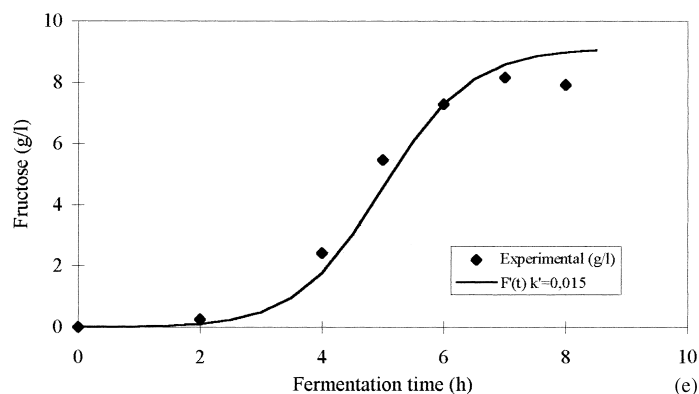


Fig. 5. (Continued)

Table 6

Model parameters  $\mu_m$ ,  $X_0$ ,  $Y_{E/X}$ ,  $Y_{X/S}$ , and  $k'$  calculated for other experiments

Run	$T$ ( $^{\circ}\text{C}$ )	pH	$S'$ (g/l)	$\mu_m$ ( $\text{h}^{-1}$ )	$X_0$ (g/l)	$Y_{E/X}$ (DSU/mgcell)	$Y_{X/S}$ (g cell/g sucrose)	$k'$ (ml/DSU h)
5	25	5.5	20	1.272	0.00654	16.64	3.41	0.015
31	35	5.5	20	1.423	0.00523	13.95	3.57	0.015
5	25	5.5	20	1.272	0.00654	16.64	3.41	0.015
4	25	6.7	20	0.748	0.09220	7.36	3.35	0.010
1	25	s. c.	20	0.808	0.00723	12.27	2.03	0.011
1	25	s. c.	20	0.808	0.00723	12.27	2.03	0.011
2	30	s. c.	20	1.037	0.00599	10.02	1.15	0.012
6	25	6.7	40	0.877	0.02860	8.13	1.10	0.010
7	25	5.5	40	0.947	0.01880	16.48	1.12	0.010
14	25	6.7	10	0.968	0.12290	3.57	2.65	0.017
4	25	6.7	20	0.748	0.09220	7.36	3.35	0.010
6	25	6.7	40	0.877	0.02860	8.13	1.10	0.010
17	25	6.7	80	0.822	0.02270	3.29	0.33	0.010
31	35	5.5	20	1.423	0.00523	13.95	3.57	0.015
20	35	5.5	80	1.306	0.00764	5.53	0.39	0.013
22	35	5.5	120	1.076	0.01432	7.18	0.27	0.014
15	25	5.5	10	1.181	0.00838	18.37	1.26	0.017
5	25	5.5	20	1.272	0.00654	16.64	3.41	0.015
7	25	5.5	40	0.947	0.01880	16.48	1.12	0.010
19	25	5.5	80	1.073	0.00962	7.14	0.28	0.010

time. The package MAPLE 5.0 and Runge–Kutta–Fehlberg of 4th and 5th order numerical method were used to solve those equations and results for  $S'$ ,  $D'$  and  $F'$  as a function of time are presented in Fig. 5c, d and e using the best value of  $k' = 0.015$  ml/DSU h.

The assumption of first order kinetics with regard to enzyme and sucrose concentrations for the production of dextran and fructose are further supported by rearranging Eqs. (10) and (11) as  $E(t)/r'_F$  and  $E(t)/r'_D$  versus  $1/S'(t)$ . Both plots are straight lines passing through the origin indicating a first order kinetics relative to the substrate concentration. From the slope we can also estimate  $1/k'$ .

Table 5 provides a summary of the kinetic model equations and input data required for the model simulations.

## 5. Discussion of results

The model proposed is a fair approximation of the experimental results obtained for the typical run #31. At the end

of the fermentation (see Fig. 5e) model results for fructose deviate from experimental data. This is due to the fact that when there is almost no sucrose, fructose is consumed by the bacteria as an alternative carbon source. Similar calculations were carried out for other experiments and results are summarised in Table 6.

The enzyme production rate is higher for pH = 5.5 and decreases as the initial sucrose concentration increases. At pH = 6.7 it seems that the enzyme production has an optimum value for sucrose initial concentration in the range 20 to 40 g/l. However, the enzyme production is always smaller than for pH = 5.5.

The enzyme is more active for lower temperatures and for pH = 5.5. At 25 $^{\circ}\text{C}$  and pH = 5.5 the yield  $Y_{E/X}$  tends to decrease for initial sucrose concentration above 40 g/l.

For both pH values of 5.5 and 6.7, the yield  $Y_{X/S}$  has an optimum value when the initial sucrose concentration is 20 g/l. This value is influenced by the temperature and the optimum value for 20 g/l of initial sucrose is at 35 $^{\circ}\text{C}$ .

The kinetic constant  $k'$  has the highest value in fermentations #14 and #15, where  $k' = 0.017$  ml/DSU h. These runs, with initial sucrose concentration of 10 g/l (very low) is extremely fast and the cells are under stress since the beginning of the fermentation due to the substrate low concentration and to the competition for substrate between cells and produced extracellular enzyme.

For the initial sucrose concentration of 20 g/l and pH = 5.5, the kinetic constant  $k'$  is 0.015 ml/DSU h. When the pH is not controlled or controlled at pH = 6.7 for the same initial sucrose concentration,  $k'$  is between 0.010 and 0.012 ml/DSU h. For pH = 5.5 and  $T = 35^\circ\text{C}$ , the kinetic parameter  $k'$  has an optimum value for initial sucrose concentration of 20 g/l.

The model prediction concerning enzyme activity shows some time lag relative to the experimental data. This can be explained by the fact that our simple model does not account for mass transport from the cell where the enzyme is produced to the external bulk liquid phase.

## 6. Conclusions

Experimental results on the batch fermentation of *L. mesenteroides* with sucrose are presented in this work. Batch fermentations carried out at  $35^\circ\text{C}$  and pH control at pH = 5.5 resulted in highest enzyme levels for sucrose concentrations up to  $40\text{ g/dm}^3$ . Dextran and fructose concentrations reach 8.17 g/l and 8.15 g/l, respectively at the end of the fermentation. For higher sucrose concentrations (80 and  $120\text{ g/dm}^3$ ) there were problems with cell centrifugation. In this step of the process, enzyme and even small quantities of fructose and dextran were centrifuged with cell mass. Aeration did not improve the fermentation process.

The model developed to simulate the conversion of sucrose to dextran and fructose gives a good description of the batch fermentation with *L. mesenteroides*. Optimum values of the kinetic constant  $k'$  were found for initial substrate concentration of 20 g/l, confirming that this is the best initial concentration of sucrose for the strain *L. mesenteroides* NRRL B512(f).

## 7. Notation

$D$	dextran concentration (mol/l)
$D'$	dextran concentration (g/l)
$E$	enzyme activity (DSU/ml)
$F$	fructose concentration (g/l)
$F'$	fructose concentration (mol/l)
$k$	kinetic parameter in Eqs. (5) and (6)
$k'$	kinetic parameter in Eqs. (11) and (12) (ml/DSU h)
$M_S$	molar mass of sucrose
$M_F$	molar mass of fructose
$M_W$	molar mass of water

$n$	stoichiometric coefficient for sucrose
$S$	sucrose concentration (mol/l)
$S'$	sucrose concentration (g/l)
$r_x$	cell growth rate (g cells/l/h)
$(-r'_S)$	substrate consumption rate (g/l/h)
$t$	time (h)
$T$	temperature ( $^\circ\text{C}$ )
$X$	Cell concentration (g/l)
$z$	stoichiometric coefficient for dextran
$Y_{E/X}$	yield enzyme/substrate
$Y_{X/S}$	yield biomass/substrate

### 7.1. Greek symbols

$\mu$	specific cell growth rate ( $\text{h}^{-1}$ )
$\mu_{\max}$	maximum specific growth rate ( $\text{h}^{-1}$ )

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