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ORIGINAL PAPER

Factors affecting the production of L-xylulose by resting cells of recombinant *Escherichia coli*

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Abstract Factors affecting the production of the rare sugar L-xylulose from xylitol using resting cells were investigated. An *E. coli* BPT228 strain that recombinantly expresses a gene for xylitol dehydrogenase was used in the experiments. The ratio of xylitol to L-xylulose was three times lower in the cytoplasm than in the medium. The effects of pH, temperature, shaking speed, and initial xylitol concentration on L-xylulose production were investigated in shaking flasks using statistical experimental design methods. The highest production rates were found at high shaking speed and at high temperature (over 44°C). The optimal pH for both productivity and conversion was between 7.5 and 8.0, and the optimal xylitol concentration was in the range 250–350 g l^{-1}. A specific productivity of 1.09 ± 0.10 g g^{-1} h^{-1} was achieved in a bioreactor. The response surface model based on the data from the shake flask experiments predicted the operation of the process in a bioreactor with reasonable accuracy.

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Introduction

In recent years, interest in L-carbohydrates and nucleosides derived from them has increased substantially in medicine. Nucleoside analogs of L-sugars are used as drugs against viruses such as hepatitis B and HIV [14]. L-Sugars can also be used to support cancer treatments. Not only do these compounds increase the mortality rates of tumor cells, but they also decrease the growth rates of malignant cells [3].

L-Xylulose (*threo*-pentulose) is a ketopentose that is rare in nature and currently very expensive. It is an inhibitor of glucosidase I and should therefore be useful as an inhibitor of glycoprotein processing in various cell culture systems [16]. An L-xylulose containing drug preparation for reducing blood sugar levels in humans and animals has been patented [10]. L-Xylulose can also be used as a precursor of other rare sugars. For example, the isomerization of L-xylulose to L-xylose either enzymatically or in an aqueous solution under alkaline conditions has been reported [9, 12]. The isomerization product, L-xylose, can be used as a starting material for the production of L-ribonucleosides, which find use in medicine. The conversion of L-xylulose to the rare sugar L-lyxose has also been described [2].

D- and L-xylulose are intermediates in many prokaryotic and eukaryotic metabolic pathways [5]. Some bacteria have been reported to metabolize xylitol via pathways in which xylitol is first oxidized to L- or D-xylulose. Natural mutants of *Pantoea ananatis* that are able to use xylitol as the sole carbon source have been isolated. These natural mutants were subjected to transposon mutagenesis to achieve xylitol-negative mutants. One of these, *P. ananatis*

ATCC43072, still constitutively synthesized xylitol-4-dehydrogenase, but lacked L-xylulokinase activity. The mutant converted xylitol to L-xylulose without phosphorylating and metabolizing it further. L-Xylulose was produced by resting cells of this strain with xylitol as the substrate [6].

It has also been reported that resting cells of the bacterium *Alcaligenes* sp 701B can be used to produce L-xylulose from xylitol [13]. The same strain has been used by Granström et al. [7] in the growth state with D-xylose as the carbon source for growth, and xylitol as the precursor of L-xylulose. In addition, a new facultatively thermophilic bacterium named *Bacillus pallidus* has recently been isolated. Resting cells of this strain were shown to produce L-xylulose from xylitol with high conversion [17].

We have previously reported the expression of the xylitol-4-dehydrogenase gene (*xdh*) of *P. ananatis* in *Escherichia coli* and the characterization of the recombinant protein. The resting cells of these recombinant cells were shown to convert xylitol solely to the L-form of xylulose [1]. In the present study, we have expressed the *xdh* gene of *P. ananatis* in *E. coli* in order to study the production of L-xylulose from xylitol using resting cells in shake flasks and in a bioreactor. We have also investigated the intracellular levels of the metabolites that take part in this reaction, and the effects of various factors on the production process.

Materials and methods

Growth conditions

The *E. coli* strains and *P. ananatis* ATCC43072 were cultivated in shake flasks at 200 rpm in LB broth at 37 and at 30°C, respectively. The rpm values in shake flasks presented in this work refer to rotary shaking with an amplitude of 2.5 cm. When required, the growth medium was supplemented with 34 mg l^{-1} chloramphenicol.

Cloning *xdh* into *Escherichia coli*

xdh was multiplied by PCR using the genomic DNA of *P. ananatis* ATCC43072 as the template. The primers XDHDuetF (5′-AAT TCA TAT GTG CGG TGA ATA TGA CG-3′) and XDHDuetR (5′-AAT TCT CGA GAT TTA CCA GAT GGT AAA G-3′) were used to insert *Nde*I and *Xho*I restriction sites (underlined) at the 5′- and 3′-ends of the *xdh* gene, respectively. The PCR product and the expression vector pACYCDuet (Novagen) were digested with *Nde*I and *Xho*I and *xdh* was ligated into the second cloning site of the vector.

Since the pACYCDuet vector requires a DE3 strain for expression, *E. coli* HB101 was subjected to DE3 lysogenization using the Novagen DE3 lysogenization kit. The pACYCDuet vector containing the *xdh* insert was transformed into the DE3-lysogenized HB101 cells [8]. An XDH-positive transformant was named *E. coli* BPT228 and selected for further study.

Expression of *xdh* and preparation of cell extracts

The *E. coli* BPT228 cells were induced with 1 mM IPTG (isopropyl-thio-β-D-galactopyranoside) at an OD_{600} value between 0.5 and 0.7 and grown for a further 3 or 4 h. The cells were collected by centrifugation at 3,000×*g* and at 4°C for 5 min. The cells were washed with cold sonication buffer containing 50 mM Tris, 50 mM glycine, NaOH, pH 9.0 and 10 mM $MgCl_2$, and resuspended in 1 ml of the sonication buffer supplemented with protease inhibitor tablets (one per 20 ml; Complete Mini, EDTA-free, Roche). The cells were disrupted by sonication and the cell debris removed by centrifugation at 28,000×*g* for 20 min at 4°C.

Intracellular metabolites

The *E. coli* BPT228 cells were grown in 85 ml batches, induced, and harvested as described above. The cells were suspended into a solution (8.5 ml) containing 154 mM NaCl, 5 mM $MgCl_2$ and 17 mg l^{-1} chloramphenicol. Xylitol and D-[1-^{3}H]xylitol (American Radiolabeled Chemicals Ltd., St. Louis, MO, USA) were added to the final concentration of 10 g l^{-1} and to the final radioactivity of 4.5 μCi l^{-1}, respectively. The cell suspensions were incubated at 37°C and at 200 rpm for 3 h. Cells were separated from the medium using a modification of a method described previously [11]. Samples (2 ml) were centrifuged through 500 μl of silicone oil (Silconöl PN200, Carl Roth, Karlsruhe, Germany) at 16,000×*g* for 1 min. The L-xylulose and xylitol concentrations of the supernatants were analyzed by HPLC as described below.

The separated cells were suspended in 2 ml of NAD^+/NADH extraction buffer (NAD^+/NADH Quantification kit, BioVision) and disrupted by vortexing in the presence of glass beads at 4°C. The cell lysate was separated and centrifuged at 20,000×*g* and at 4°C for 20 min. An aliquot (100 μl) of the resulting cell extract was used for the quantification of intracellular pyridine nucleotides using the Biovision NAD^+/NADH quantification kit. The rest of the cell extract was neutralized with HCl to prevent L-xylulose degradation, and the precipitate formed was separated by centrifugation at 16,000×*g* for 2 min. The samples were stored at −80°C until further analysis of intracellular molar ratios of xylitol to L-xylulose.

The samples were filtered through 0.2 μm membranes and subjected to hydroxyl affinity chromatography in order to separate the L-xylulose and xylitol. The samples were

applied to a column (2.6 by 135 cm) of Finex CS11 GC (Finex, Kotka, Finland) in the Ca^{2+} form. The column was eluted with deionized water at an elution rate of 5 ml min^{-1}. Fractions containing L-xylulose and xylitol were collected and concentrated by evaporation under reduced pressure. The amount of radioactivity in the fractions was determined by scintillation counting (Wallac 1409, Turku, Finland).

As a control, *E. coli* BPT228 cells were incubated in the absence of xylitol, and the intracellular molar ratios of NAD^+ to NADH were determined as described above.

The effect of environmental conditions on L-xylulose production

The effects of different factors on L-xylulose production from xylitol using resting cells at high cell density were investigated using experimental designs. The effects of pH, shaking speed, and xylitol concentration and their interactions were studied using a central composite circumscribed (CCC) design. pH was varied between 5.0 and 9.0 with star points at 3.6 and 10.4, and the shaking speed was varied between 100 and 250 rpm with 49 and 300 rpm used as the star points. The initial xylitol concentration was varied logarithmically between 10 and 100 g l^{-1}, with star points at 4.6 and 219 g l^{-1}. This resulted in an experimental design comprising 17 experiments. For this, the cells were grown in 9 l, induced, and harvested as described above. The cells were washed with double-strength reaction buffer without xylitol. The cells were incubated in the reaction buffer (25 ml) containing 50 mM citrate, 50 mM Tris, 10 mM $MgCl_2$ and 34 mg l^{-1} chloramphenicol at different pH values and xylitol concentrations. The reaction was continued for 48 h at 37°C, and 1 ml samples were taken for HPLC analysis. pH values of the samples were estimated using pH-indicator strips.

The effects of temperature, pH and initial xylitol concentration and their interactions on L-xylulose production by resting cells of *E. coli* BPT228 were further studied using a central composite face-centered (CCF) design. 50 mM K phosphate–50 mM Tris buffer supplemented with 5 mM $MgCl_2$ and 17 mg l^{-1} of chloramphenicol were used at the pH values 7.0, 7.75 and 8.5. Temperatures ranged from 36 to 44°C in the experiments. The initial xylitol concentration was varied between 150 and 450 g l^{-1}. In addition, four extra experiments were performed at the star points of pH and xylitol concentration (star distance 1.5). This resulted in an experimental design of 21 experiments.

The cells were grown and induced at 5 l as described above. The cells were washed with a solution containing 154 mM NaCl and 5 mM $MgCl_2$ and suspended in this solution. Each reaction was started by inoculating the reaction mixtures with the cell suspension, resulting in an initial cell density of 4.9 g l^{-1} (cell dry weight). The cell suspensions (25 ml) were incubated in 250 ml Erlenmeyer flasks with shaking at 300 rpm. Samples (1 ml) were taken for HPLC analysis. The pH values were measured from each sample at the reaction temperature using a pH electrode.

The results were evaluated using statistical coefficients: coefficient of determination (R^2), coefficient of model prediction (Q^2), *P* value, and *P* for the lack of fit. These values indicate how well the model corresponds to the observations (R^2), how well the model is able to predict experimental results (Q^2), and how valid the model is (*P* values). The experimental designs were made and evaluated using Modde 5.0 (Umetri, Sweden). The optimum values were predicted with a prediction tool provided by Modde.

L-Xylulose production in a bioreactor

L-Xylulose production was investigated in a Biostat MD reactor (total volume 2 l, B. Braun Biotech International) equipped with two six-blade Rushton-type impellers. An overnight culture (500 ml) of *E. coli* BPT228 was used to inoculate 1.3 l of LB medium. The temperature was set at 40°C and the pH at 8.0. Dissolved oxygen was controlled at a minimum of 30% air saturation by varying the stirring rate (minimum 400 rpm) and aeration rate (minimum 0.5 vvm). After 1 h of cultivation, the expression of *xdh* was induced by the addition of IPTG to the final concentration of 1 mM. The cells were cultivated for an additional 3.5 h after the induction.

Cells were concentrated by tangential flow filtration using a Pellicon Mini (Millipore, Billerica, MA, USA) holder and two Biomax 1,000 K membranes (total filtration area 0.2 m^2, Millipore). The cells were resuspended into a solution (1 l) containing 50 mM K phosphate–50 mM Tris-HCl buffer pH 8.0, 5 mM $MgCl_2$ and 250 g l^{-1} xylitol. Production of L-xylulose with resting cells was carried out in the bioreactor at 40°C for 24 h. The pH was maintained at 8.0 through the addition of 3 M NaOH. Dissolved oxygen was controlled at a minimum of 30% air saturation, as described above. The samples taken were analyzed by HPLC for L-xylulose, as described below.

Activity assays

The XDH activity assays were carried out at 30°C by measuring the change in absorbance at 340 nm. The reaction mixture contained 50 mM Tris, 50 mM glycine, NaOH pH 9.0, 10 mM $MgCl_2$, 500 mM xylitol, and 1 mM NAD^+. Protein concentrations were determined using the Bio-Rad protein assay reagent with bovine serum albumin as the protein standard.

HPLC

The samples for HPLC were centrifuged at 16,000×*g* and at room temperature for 1 min and filtered through a 0.2 μm membrane. A Waters 410 refractive index detector was used in all HPLC analyses. L-Xylulose and xylitol analyses were performed using an HPX-87K column (Bio-Rad) and a K precolumn. Water was used as the eluent at 85°C at a flow rate of 0.4 ml min^{-1}, except when samples from the incubations in borate buffer were analyzed, in which case the eluent was 16 mM boric acid titrated to pH 8.5 with NaOH [4].

Results

Cloning of *xdh* into *Escherichia coli*

E. coli HB101(DE3) cells were transformed with the vector pACYCDuet inserted with the *xdh* gene. The transformants were tested for XDH activity and for the ability to convert xylitol to L-xylulose in the resting state. The clone that showed the highest xylitol dehydrogenase activity of 0.75 ± 0.20 μmol s^{-1} g^{-1} and converted xylitol to L-xylulose quantitatively was named BPT228 and selected for further studies.

Intracellular metabolites

The extracellular concentrations of L-xylulose and xylitol and the intracellular molar ratios of xylitol to L-xylulose and NAD^+ to NADH were analyzed at 1 and 3 h from suspensions of resting cells of *E. coli* BPT228 incubated in the presence of radiolabeled xylitol (10 g l^{-1}). As a control, *E. coli* BPT228 was incubated in the absence of xylitol and the intracellular molar ratios of NAD^+ to NADH were determined.

Despite the complexity of the procedure, reasonably low standard errors (less than 25%) were achieved in the experiments performed in triplicate. Estimates for the intracellular molar ratios of xylitol to L-xylulose were 4.7 at 1 h and 4.1 at 3 h. The corresponding extracellular ratios were 14 at 1 h and 13 at 3 h. The results show that the intracellular ratio of xylitol to L-xylulose was lower (over three times lower) than the extracellular ratio. The presence of xylitol had no effect on the intracellular ratio of NAD^+ to NADH (10 with xylitol and 8 without it at 1 h). However, for an unknown reason the NAD^+ to NADH ratio nearly doubled between the two time points. Apparently the NAD^+ is efficiently regenerated despite the dehydrogenation reaction. The total amount of xylitol and L-xylulose inside the cells was virtually constant during the experiment (data not shown).

Effects of environmental conditions on L-xylulose production

A CCC experimental design was used to investigate L-xylulose production by resting cells of *E. coli* BPT228 at a high cell density after 12 h of incubation. Temperature was held constant at 37°C, with pH, shaking speed and initial xylitol concentration used as the variables. 50 mM citrate–50 mM Tris buffer was used to achieve a pH range that was as broad as possible. The experimental design and the results are presented in Table 1, and the response surface models for conversion and productivity are presented in Fig. 1. The model for conversion showed a coefficient of determination (R^2) of 0.933, a coefficient of model prediction (Q^2) of 0.716, and a *P* value of less than 0.001. The *P* value for the lack of fit was 0.145. These statistical coefficients indicate that the model is highly significant and able to predict conversion at a certainty level of 72%. The model for productivity showed an R^2 of 0.941, a Q^2 of 0.828, and a *P* value of less than 0.001. The *P* value for the lack of fit was 0.103. These statistical coefficients indicate that the model is again highly significant and able to predict productivities at a certainty level of 83%. In both models the effect of shaking speed was positive but very small. According to the models, a nearly neutral pH (pH 7.7) was optimal for both

Table 1 Central composite circumscribed (CCC) experimental design for L-xylulose conversion and productivity with xylitol concentration, pH and shaking speed used as the variables

Factors			Responses	
Xylitol (g l^{-1})	pH	Shaking (rpm)	Conversion (mol mol^{-1})	Productivity (g l^{-1} h^{-1})
10.1	5.0	100	0.37	0.31
9.8	9.0	100	0.56	0.45
8.6	5.0	250	0.48	0.34
8.8	9.0	250	0.59	0.43
98.4	5.0	100	0.14	1.14
100.8	9.0	100	0.21	1.73
94.7	5.0	250	0.16	1.23
96.4	9.0	250	0.22	1.72
31.2	3.6	175	0.02	0.05
31.7	10.4	175	0.10	0.26
32.4	7.0	49	0.28	0.30
32.3	7.0	301	0.34	0.91
4.4	7.0	175	0.84	0.31
208.2	7.0	175	0.15	2.60
32.1	7.0	175	0.34	0.91
36.1	7.0	175	0.30	0.90
37.1	7.0	175	0.11	0.85

The xylitol concentrations presented were measured from the experiments

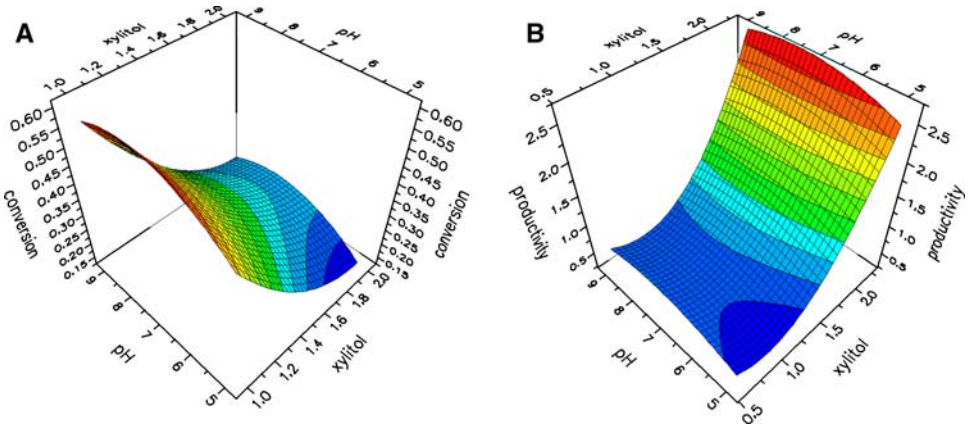

Fig. 1 Response surface models of the effects of pH and xylitol concentration on **a** conversion and **b** productivity with resting cells of *E. coli* BPT228. Xylitol concentration is presented on a log scale. The shaking speed was 250 rpm in both models

conversion and productivity. As expected, a low xylitol concentration (10 g l^{-1}) was optimal for achieving high L-xylulose conversion. On the other hand, productivity was higher at high initial xylitol concentrations. The xylitol concentration and pH showed no significant interaction effects on either conversion or productivity. Since the productivity optimum was outside the investigated region of xylitol concentration, we used another experimental design with a different range of xylitol concentrations.

The effects of temperature, pH and initial xylitol concentration on L-xylulose production by resting cells of *E. coli* BPT228 were investigated using a CCF experimental design with additional experiments for the star points of pH and initial xylitol concentration. The time points at which the concentration of xylitol increased by 7% per h were determined for each experiment. The effects of the environmental factors on the conversion and on the volumetric productivity were investigated at these time points. The experimental design and results are shown in Table 2. The response surface models for conversion and productivity are shown in Fig. 2. The model for the conversion had one outlier: the experiment performed at the lower xylitol concentration star point. When this outlier was removed, the R^2 for the model was 0.995, the Q^2 was 0.990, and the P value was less than 0.001. The P value for the lack of fit was 0.238. These statistical coefficients suggest that the model was highly significant and capable of predicting conversion at a certainty level of 99%. The model for productivity had an R^2 value of 0.977, a Q^2 value of 0.942, and a P value of less than 0.001. The P value for the lack of fit was 0.337. These statistical coefficients suggest that the model was highly significant and capable of predicting productivity at a certainty level of 94%. The effect of pH was very small or negligible in these models. The results suggested that the temperature optimum for productivity would be outside the investigated area (over 44°C). Temperature affected the productivity significantly but had very little effect on conversion. The combined effects of pH and temperature on both productivity and conversion were found to be insignificant. Xylitol concentration showed an optimum for productivity at around 350 g l^{-1}.

Table 2 Experimental design for investigating the effects of xylitol concentration, pH and temperature on L-xylulose conversion and productivity

Factors			Responses	
Xylitol (g l^{-1})	pH	Temperature (°C)	Conversion (mol mol^{-1})	Productivity (g l^{-1} h^{-1})
142.1	7.33	40	0.175	2.98
447.0	7.26	40	0.065	3.51
287.7	6.87	40	0.110	4.15
295.1	7.70	40	0.109	4.05
297.6	7.28	40	0.107	3.83
290.4	7.28	40	0.111	3.94
286.4	7.26	40	0.115	4.04
73.6	7.33	40	0.245	1.64
476.0	7.23	40	0.061	3.18
257.9	6.57	40	0.118	3.86
293.0	7.75	40	0.107	3.94
145.6	6.91	44	0.169	3.58
439.5	6.81	44	0.080	4.21
140.5	7.54	44	0.174	3.20
440.5	7.68	44	0.080	4.41
289.9	7.24	44	0.113	4.97
132.4	6.94	36	0.171	2.88
417.8	6.83	36	0.064	3.18
142.3	7.61	36	0.162	2.79
437.5	7.70	36	0.067	3.15
290.6	7.31	36	0.103	3.65

The xylitol concentrations and pH values presented were determined from the experiments

Scale-up experiment in a bioreactor

The scale-up experiment was performed in duplicate as described in "Materials and methods." A temperature of

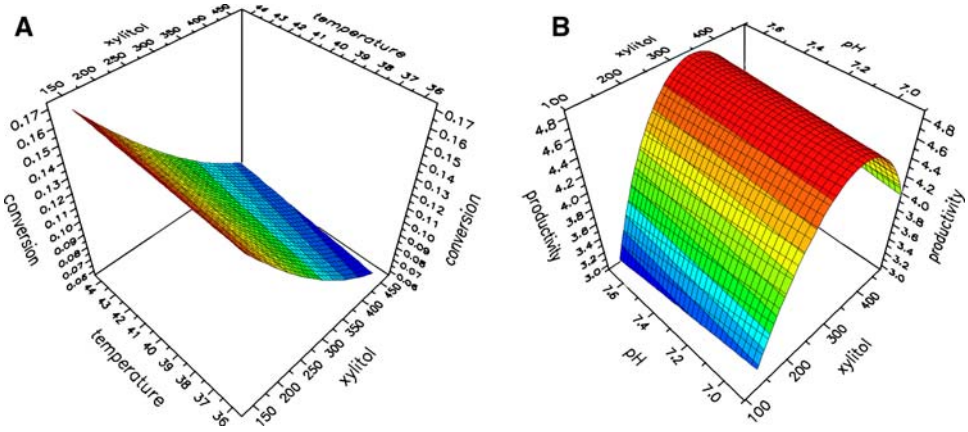

Fig. 2 Response surface models of the effects of temperature, pH and xylitol concentration on **a** conversion (the effect of pH was negligible) and **b** productivity (temperature 44°C) with resting cells of *E. coli* BPT228

40°C was chosen for the scale-up experiment because the poor stability of the L-xylulose produced at pH 8.0 and elevated temperatures could have negative effects on productivity (data not shown). The initial cultivation phase yielded 0.8 g l^{-1} cells (dry weight) during the 4 h cultivation. The cell concentration phase lasted approximately 15 min and the washing phase 10–15 min. Thus, the production experiment was started approximately 30 min after the completion of cultivation. The L-xylulose concentrations during the production phase are shown in Fig. 3. The productivity at the 7% hourly L-xylulose increase was 1.50 ± 0.14 g l^{-1} h^{-1}. The model presented above predicted a total productivity of 3.76 ± 0.19 g l^{-1} h^{-1}. However, the cell density was significantly different in the bioreactor compared to the modeling experiments. The corresponding specific productivity of 1.09 ± 0.10 g g^{-1} h^{-1} obtained in the bioreactor corresponds reasonably well with the specific productivity of 0.77 ± 0.04 g g^{-1} h^{-1} predicted by the model. The results suggest that scaling up this process would be a straightforward task.

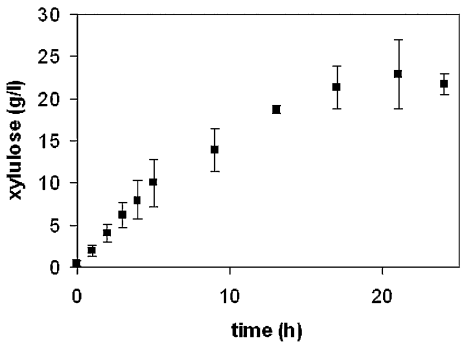

Fig. 3 L-Xylulose concentrations in the scale-up experiment with resting cells of *E. coli* BPT228. The *error bars* represent standard deviations calculated from replicate experiments

Discussion

Using resting cells for L-xylulose production has several advantages over other methods. Compared to the chemical procedure reported in [18], only minor amounts of by-products are formed and the yields are much higher. The regeneration of cofactors by the intact cells and the general stability of intracellular enzymes make this production mode more favorable than procedures based on the use of isolated enzymes. Furthermore, the L-xylulose produced can be easily separated and purified from the production medium.

Unfortunately, the performances of the different production strains described in the literature cannot be properly compared, since no data are available on the specific productivities. The only exception is the specific productivity of 0.21 g g^{-1} h^{-1} obtained using resting cells of *Alcaligenes* sp. 701B at an initial xylitol concentration of 5 g l^{-1} [13]. This productivity is lower than the specific productivities of 1.09 and 0.33 g g^{-1} h^{-1} obtained in the present work at 250 and at 70 g l^{-1} of xylitol, respectively.

Poonperm et al. [17] described the production of L-xylulose from xylitol using the cells of a newly isolated facultative thermophilic bacterium *Bacillus pallidus* Y25 in the resting state. The volumetric productivity of 2.3 g l^{-1} h^{-1} achieved using this strain at 100 g l^{-1} xylitol is the highest of those previously reported. The authors state that the conversion was carried out at low cell density, since the L-xylulose was consumed by the cells at higher densities. However, high cell densities would be preferable in terms of maximizing the volumetric productivity on an industrial scale. Furthermore, the biotransformation using the *B. pallidus* strain was carried out at pH 9.0 and 50°C. Our preliminary results show that high temperature in combination with high pH may be problematic in an industrial process, since L-xylulose is unstable under these conditions (data not shown).

In our previous study, we reported that xylitol exhibits substrate inhibition on xylitol-4-dehydrogenase activity at concentrations of over 15 g l^{-1} [1]. Therefore, we chose initial xylitol concentration as a variable in the factorial studies. However, the optimum initial xylitol concentration for a productivity of approximately 350 g l^{-1} (Fig. 2) was near the highest xylitol concentration (400 g l^{-1}) that did not affect cell viability (data not shown). The previously reported results and the results of the current study suggest that higher conversions can be obtained at lower xylitol concentrations. As a compromise between productivity and conversion, the scale-up experiment in the 2 l bioreactor was conducted at 250 g l^{-1} of initial xylitol.

For practical reasons, the effects of the chosen parameters on L-xylulose production were studied in shake flasks. Although it was not possible to control the level of dissolved oxygen in the flasks, the model based on the data from these experiments predicted the productivity of the cells in the bioreactor with controlled aeration fairly well. Most likely, the lack of oxygen was not limiting to the production since the cells were in a non-growth state. We have estimated the ratios of the reactants that take part in the dehydrogenation of xylitol to L-xylulose in the current work. We have previously reported that the xylitol dehydrogenase has a very low activity for $NADP^+$ and that NAD^+ is the preferred substrate [1]. The reaction did not appear to affect the intracellular balance of NAD^+ to NADH to any significant extent, as the comparison between the resting cells in the presence and absence of extracellular xylitol shows. It seems that the NAD^+ consumed in xylitol dehydrogenation is efficiently regenerated by the cells under the conditions used.

Aeration is required for the dehydrogenation of xylitol to L-xylulose by the resting *E. coli* BPT228 cells (data not shown). The NAD^+ consumed in the reaction has to be regenerated by the cells for the L-xylulose production to continue. Under aerobic conditions, *E. coli* cells use NADH dehydrogenases from their respiratory chains for NAD^+ regeneration, with oxygen being the final electron acceptor. NADH oxidases are possibly also used for NAD^+ regeneration by the cells. Under anaerobic conditions and in the absence of alternatives to oxygen, NAD^+ can only be regenerated in reactions in which metabolic intermediates are reduced [15, 20]. No external carbon sources were available in the medium from which such metabolic intermediates could be formed.

High conversions of xylitol to L-xylulose (>70%) have previously been achieved only at low xylitol concentrations. A low initial concentration of xylitol would demand high production volumes, as the volumetric productivities are low. However, if high concentrations are used, the conversion of xylitol remains low. The unreacted xylitol would therefore have to be separated and recycled. L-Xylulose has been previously recovered from the bioconversion media by ion-exclusion chromatography [1, 6, 7, 13, 17]. If the bioconversion medium contains high concentrations of xylitol, the solution should be enriched in L-xylulose prior to chromatographic separation. The downstream processing is currently under investigation in our laboratory. The recyclability of the cells should also be investigated, since this could result in savings in the cost of the medium [19].

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