HAL
open science

Improvement of a fed-batch process for high level xylanase production by a Bacillus strain

Gerhard Schneider, Pierre Strehaiano, Patricia Taillandier

To cite this version:

Gerhard Schneider, Pierre Strehaiano, Patricia Taillandier. Improvement of a fed-batch process for high level xylanase production by a Bacillus strain. Journal of Chemical Technology and Biotechnology, 2001, 76 (5), pp.456-460. 10.1002/jctb.415 . hal-02135100

HAL Id: hal-02135100

https://hal.science/hal-02135100

Submitted on 21 May 2019

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Open Archive Toulouse Archive Ouverte (OATAO)

OATAO is an open access repository that collects the work of some Toulouse researchers and makes it freely available over the web where possible.

This is an author's version published in: https://oatao.univ-toulouse.fr/22844

Official URL : https://doi.org/10.1002/jctb.415

To cite this version :

Schneider, Gerhard and Strehaiano, Pierre and Taillandier, Patricia *Improvement of a fed-batch process for high level xylanase production by a Bacillus strain.* (2001) Journal of Chemical Technology & Biotechnology, 76 (5). 456-460. ISSN 0268-2575

Any correspondence concerning this service should be sent to the repository administrator: tech-oatao@listes-diff.inp-toulouse.fr

Improvement of a fed-batch process for high level xylanase production by a *Bacillus* strain

Gerhard Schneider, Pierre Strehaiano and Patricia Taillandier*
INP - ENSIGC, Laboratoire de Génie Chimique, UMR-CNRS 5503, 18, Chemin de la Loge, 31078 Toulouse cedex 4, France

Abstract: In this paper, the improvement of a fed-batch fermentation from the point of view of an industrial xylanase production process is described. The *Bacillus* strain chosen for this study is able to produce high quantities of a xylanase that is suitable to be used as bleach boost agent in chlorine-free bleaching sequences of paper pulp. It was found that xylo-oligosaccharides (hydrolysis products from xylan by xylanase action) were indispensable for induction of the enzyme synthesis, but that their presence in quantities of only 0.1 g dm^{-3} xylose equivalents led to catabolite repression. A substrate-limited fed-batch process, that is the most adapted, was furthermore improved with regard to nutrient requirement of the microorganism, especially the nitrogen source. A process with constant supply of a culture medium containing xylan, peptone and mineral nitrogen was able to produce 20240 nkat cm^{-3} with a productivity of 910 nkat $cm^{-3}h^{-1}$, which places the process among the best ever reported.

Keywords: *Bacillus* sp; xylanase; fed-batch; catabolite repression; nitrogen source

1 INTRODUCTION

Xylanolytic enzymes from microbial sources have received increasing attention in the last 10 years, as they possess potentially valuable uses in the hydrolysis of xylan in various industrial applications. Currently, the most important application from an ecological and economic point of view is the prebleaching of paper pulp. This leads to a considerable reduction of the use of chlorine compounds, which generate environmentally harmful chlorinated organic substances.[1,2] Moreover, xylanase treatment of paper pulp allows higher target brightness levels to be reached. Xylanases can also be used for enzymatic conversion of agro-industrial residues into fermentation substrates, selective degradation of xylan in food, animal feed and in fibre technology or clarification of juices and wines.[3]

The *Bacillus* strain chosen for this study has been reported to be an efficient producer of an endo-/J(1,4)-xylanase (EC 3.2.1.8) that is suitable for the pulp and paper industry.[4] This enzyme is devoid of cellulolytic activity, thus preventing hydrolysis of cellulose fibres, and it is stable and active at a temperature up to 60 °C and pH between 5 and 7. It has also been successfully applied in a totally chlorine-free bleaching sequence.[5] For enzyme synthesis, this *Bacillus* strain requires xylo-oligosaccharides (partially hydrolysed xylan from xylanase action) as inducer. A batch culture with xylan as substrate, where a small constitutive xylanase activity generates the inducer, is able to produce up to 2750 nkat cm^{-3} in 32 h.[6] Samain *et al* showed that the enzyme production was also catabolically-repressed when the xylo-oligosaccharides accumulated in the medium. They proposed a substrate-limited fed-batch process with a constant feeding rate of xylo-oligosaccharides that was able to produce up to 16666 nkat cm^{-3}.[7]

Although catabolite repression of xylanase synthesis by monosaccharides or xylo-oligosaccharides is common among many xylanase-producing microorganisms, the improvement of the enzyme production and productivity by fed-batch or continuous cultures has rarely been applied. The first step of the present study was undertaken to obtain more detailed information about the mechanism of enzyme synthesis, considering in particular the constraints due to the catabolite repression, in order to achieve a suitable production process. The second step of this study consisted of improving the enzyme production of the fed-batch process by selection of a feeding solution to satisfy the nutrient requirement of the bacteria for maximal enzyme synthesis.

In earlier work, it was found that the presence of carbon dioxide at concentrations higher than 0.034% (concentration in air) is an essential growth factor for this *Bacillus* strain.[8] In addition, feeding of batch cultures with air enriched with 1% CO_2 had a stimulant effect on metabolic activity and cell development, as the length of biomass production was reduced by more than a half compared with a fermentation fed with air only.[9] Consequently, all cultures described in this paper were fed with carbon dioxide-enriched air in order to satisfy this particular nutrient requirement.

* Correspondence to: Patricia Taillandier, INP - ENSIGC, Laboratoire de Génie Chimique, UMR-CNRS 5503, 18, Chemin de la Loge, 31078 Toulouse cedex 4, France
E-mail: Patricia.Taillandier@ensigct.fr
Contract/grant sponsor: European Union

2 EXPERIMENTAL

2.1 Microorganism

The *Bacillus* sp I-1018 used in this study was obtained from the 'Collection Nationale de Cultures de Microorganismes (CNCM)' of the Pasteur Institute Paris, France. Cellular suspensions of the strain grown for 18 h in culture medium described below were stored at −18 °C with addition of 10% glycerol (v/v).

2.2 Culture medium

The culture medium described here was used for strain storage, inoculum growth, and fermenter cultures. Its composition was (in g dm^{-3}): soluble xylan from oat spelts 3.16 (or glucose 2 as stated in the section 3), peptone from pancreatically-digested casein (Merck no 1.02239.0500) 1.94, NaH_2PO_4-$2H_2O$ 0.6, $MgSO_4$-$7H_2O$ 0.25, K_2HPO_4 2.3, NH_4Cl 0.8, and 10 cm^3 each of trace element and vitamin solutions. Soluble xylan was prepared as follows: A suspension of xylan from oat spelts (Sigma) was treated for 15 min with ultrasound (35 kHz) and then autoclaved at 121 °C for 30 min. After decantation of the insoluble part (after 2 days at least), the supernatant was recovered by pumping and the xylan concentration was adjusted for the fermentation. The composition of the trace element solution in g dm^{-3} was: nitrilotriacetic acid 12.8, $FeCl_3$ - $6H_2O$ 1.35, $MnCl_2$ - $4H_2O$ 0.1, $CoCl_2$ - $6H_2O$ 0.024, $CaCl_2$ - $2H_2O$ 0.1, $ZnCl_2$ 0.1, $CuCl_2$ - $2H_2O$ 0.025, H_3BO_3 0.01, Na_2MoO_4 - $2H_2O$ 0.024, NaCl 1, $NiCl_2$ - $6H_2O$ 0.12, Na_2SeO_3-$5H_2O$ 0.026. The vitamin solution was prepared as follows (in mg dm^{-3}): $_D$-biotin 2, folic acid 2, pyridoxine-HCl 10, thiamine-HCl 5, riboflavin 5, nicotinic acid 5, $_{DL}$-calcium pantothenate 5, vitamin B12 0.1, *p*-aminobenzoic acid 5, lipoic acid 5. The pH was adjusted to 8 with NaOH (1 $_N$) prior to autoclaving at 121 °C for 20 min.

2.3 Preparation of xylo-oligosaccharides

Soluble xylan, prepared as described above (Section 2.2), was subjected to enzymatic hydrolysis at 50 °C for 24 h by a xylanase activity of about 1.5 nkat cm^{-3}. Reducing sugar analysis showed that hydrolysis could be considered as maximal after this period. The solution was sterilised at 121 °C for 15 min before serving for culture feeding.

2.4 Preparation of inoculum

Shake flasks (250 cm^3) containing 50 cm^3 of culture medium were seeded with 4 cm^3 of thawed stored cell suspension and incubated (50 °C, 250 rpm) for 18 h leading to a cell concentration of around 10^9 bacteria cm^{-3}. The fermenter was seeded with inoculum to give an initial concentration of about 3×10^6 bacteria cm^{-3} (determined by counting on a 'Petit-Salumbeni' haemocytometer).

2.5 Fermenter and culture conditions

Fermentations were carried out in a 5 dm^3 Inceltech type Discovery Series 210 fermenter at a temperature of 50 °C. The initial volume of the batch cultures was 2.5 dm^3. The cultures were sparged with air enriched with CO_2 (1%) at a flow rate of 1 dm^3 min^{-1}. Dissolved oxygen level was measured by an oxygen probe (Ingold) and controlled (10% of saturation) by agitation speed. The outlet gas stream first passed through a drier and then through a module (Servomex infrared) for on-line measurement of CO_2 content. Foaming was avoided by addition of 'Antifoam 289' (Sigma). For the fed-batch stage of the cultures, a peristaltic pump (Gilson) was used. Samples of the culture broth were taken every 1.5 h.

2.6 Analytical methods

Microbial growth was followed by dry weight measurement. For that purpose, a 50 cm^3 sample of the culture broth was centrifuged at 14000 rpm for 15 min. The pellet was dried in the centrifugation tube and weighed (the coefficient of variation of this method was 0.3%). On the figures, the total quantity of accumulated biomass in the fermenter was presented. The supernatant liquid was collected and stored at −20 °C until analysis. Ammonia concentration was determined by the Nessler method. Reducing sugar and glucose concentrations were analysed by the dinitrosalicylic acid (DNS) method with xylose or glucose as standard respectively.[10] Xylanase activity in the culture medium was assayed by measuring the reducing sugars liberated from birchwood xylan at 60 °C and pH 5.8 using Robinson-Britton universal buffer. Liberated reducing sugars were determined by the DNS method with xylose as standard.[10] One nanokatal (nkat) corresponds to the quantity of enzyme required to liberate one nanomole of xylose equivalent per second (the coefficient of variation of this method was 1.4%).

3 RESULTS

3.1 The enzyme synthesis mechanism

The batch stage of the two fermentations presented in this section was carried out with glucose as carbon source. After glucose exhaustion, one culture was fed with a solution of soluble xylan, and the other with a solution of already-hydrolysed xylan (xylo-oligosaccharides). This would permit production of biomass on a cheap monosaccharide and furthermore allow more detailed information about the enzyme synthesis mechanism (induction/catabolite repression) to be obtained. The aim of these experiments was also to investigate if the xylo-oligosaccharide solution could be replaced by xylan. In fact, the previous hydrolysis of large quantities of xylan is uneconomic in an industrial process.

The fermentation presented in Figs 1(A) and 2(A) was fed with a solution containing 4.5 g dm^{-3} xylan with a constant feeding rate of 1.35 g h^{-1}. The end of the growth phase, and thus the beginning of the fed-batch stage, was clearly marked after 7.5 h by a sudden fall of carbon dioxide production (Fig 1(A)) and glucose exhaustion (Fig 2(A)). During the first 3 h of

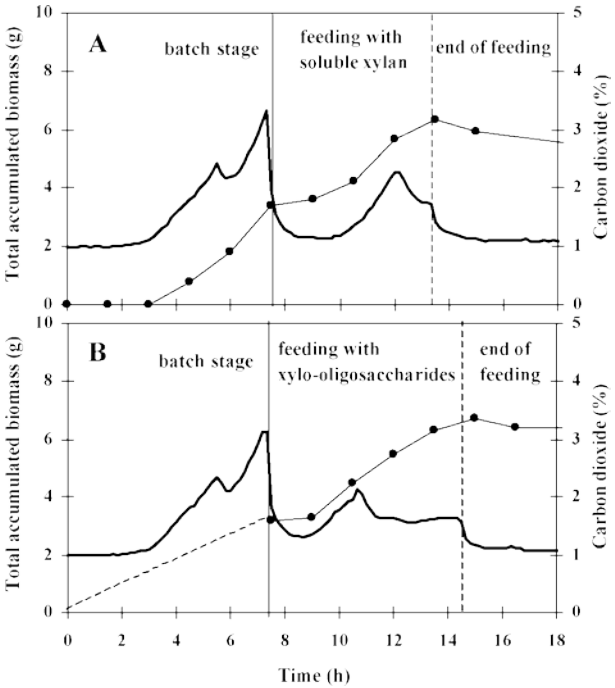

Figure 1. Accumulated biomass (•), and carbon dioxide content in the outlet gas (–) of the fed-batch culture with a feeding rate of 1.35 g h^{-1} soluble xylan (A); 1.35 g h^{-1} xylo-oligosaccharides (B).

feeding, biomass growth slowed down and carbon dioxide production almost ceased (its concentration in the inlet gas was 1%). At the same time, the concentration of reducing sugar increased in the medium (Fig 2(A)). The supplied xylan was hydrolysed into xylo-oligosaccharides by a constitutive xylanase activity of 0.3 nkat cm^{-3}, produced during growth on glucose. Obviously, during these first 3 h of feeding, the bacteria had to adapt their metabolism for the

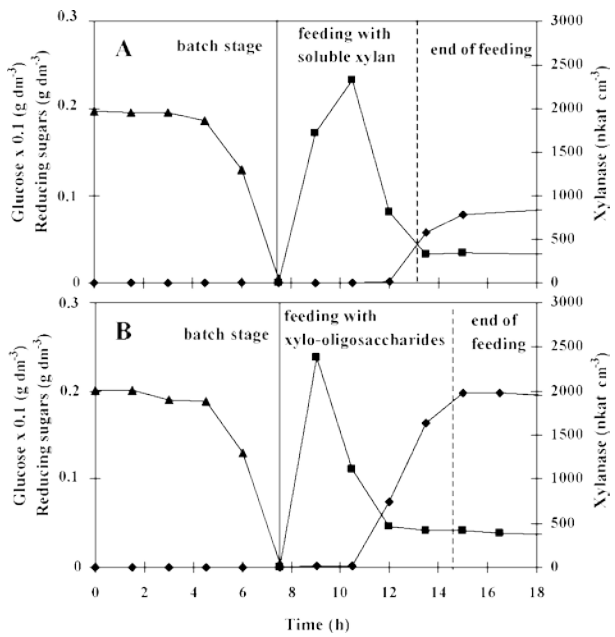

Figure 2. Glucose consumption (_.), evolution of reducing sugars (■), and xylanase production (♦) of the fed-batch culture with a feeding rate of 1.35 g h^{-1} soluble xylan (A); 1.35 g h^{-1} xylo-oligosaccharides (B).

consumption of these xylo-oligosaccharides. Growth, carbon dioxide production and sugar consumption started again after the third hour of the fed-batch stage. After consumption of accumulated reducing sugars, carbon dioxide production diminished and stabilised at a constant level during the last half-hour of the fed-batch stage. During this stationary phase, when reducing sugar concentration was below 0.1 g dm^{-3} xylose equivalents (Fig 2(A)), it can be assumed that all xylo-oligosaccharides liberated from xylan were instantaneously consumed.

The culture conditions of the fermentation presented in Figs 1(B) and 2(B) were identical to fermentation A, except that the feeding solution contained 4.5 g dm^{-3} already-hydrolysed xylan (xylo-oligosaccharides). These are directly consumable by the bacteria and so loss of time for hydrolysis of xylan by the small constitutive xylanase activity could be avoided. Figure 1(B) shows that in this case the slow down of growth and carbon dioxide production lasted only 1.5 h. During this period, the reducing sugar concentration first increased, confirming the bacteria's need of time for its metabolism to adapt to consume xylo-oligosaccharides. After this adaptation phase, accumulated sugars were consumed in 3 h. Then the culture entered a stationary state where all sugar supplied was consumed and the carbon dioxide production rate was constant. Like in the first fermentation, xylanase production started only when the reducing sugar concentration had fallen below 0.1 g dm^{-3}. The reducing sugar concentration of 0.05 g dm^{-3} observed during the stationary state was probably due to higher xylo-oligosaccharides that are not consumable by the microorganisms (Fig 2(B)).

The catabolite repression of xylanase synthesis by xylo-oligosaccharides at a concentration above 0.1 g dm^{-3} xylose equivalents has also been observed in batch cultures on xylan: xylanase production only started in the stationary phase when xylo-oligosaccharides were almost exhausted (data not shown).

3.2 Improvement of xylanase production in fed-batch cultures

The batch stages of the three cultures discussed in this section have been realised on the culture medium with soluble xylan as carbon source, to avoid metabolism change and to start enzyme synthesis immediately at the end of the batch stage. Furthermore, the aim was to lengthen the enzyme production phase by selection of nutrient feed, especially the nitrogen sources. In view of an industrial application, the idea was to produce xylo-oligosaccharides from xylan in the fermenter during the fermentation itself. For this purpose, the beginning of feeding was delayed for 1-1.5 h from the end of the growth phase. This would allow bacteria to produce a sufficient quantity of xylanase, which is able to hydrolyse xylan instantaneously when it is supplied to the fermenter in the fed-batch stage. Only the fed-batch stages of these fermentations are presented in Fig 3.

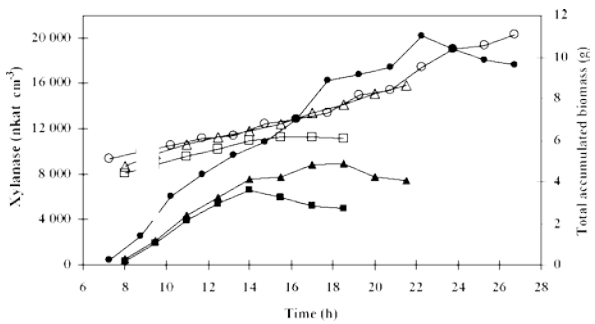

Figure 3. Accumulated biomass (open symbols) and xylanase production (closed symbols) of the fed-batch cultures with a feeding rate of: 1.35 g h^{-1} soluble xylan (■, □); 1.35 g h^{-1} soluble xylan + 68 mg h^{-1} mineral nitrogen (_., ▲); and 1.08 g h^{-1} soluble xylan + the other components of the culture medium in the usual proportions (•, o). Batch stages not shown on the figure.

The first culture has been fed with a solution containing 4.5 g dm^{-3} of soluble xylan and a constant feeding rate of 1.35 g h^{-1}. It was shown that growth and xylanase synthesis started immediately with the feeding of the fermenter. At the beginning of the feeding, bacteria had already produced 340 nkat cm^{-3} which is sufficient for instantaneous hydrolysis of the supplied xylan. In the first 6 h of feeding, the culture synthesised xylanase with a constant productivity. Xylanase production and growth ceased after the 14th hour, coincident with the exhaustion of the mineral nitrogen source, as ammonia analysis revealed (data not shown). It should also be emphasised here, that reducing sugar concentration remained below 0.05 g dm^{-3} xylose equivalents during the xylanase synthesis period (data not shown).

The second culture was fed with a solution containing xylan and NH_4Cl in order to determine if the supply of a mineral nitrogen source can extend growth and enzyme synthesis. The ammonia concentration in the feeding solution was calculated on the base of its consumption during the former fermentation. The total quantity of mineral nitrogen in the fermenter at the beginning of the fed-batch stage was 405 mg, which was consumed in 6 h. Consequently, the concentration of NH_4Cl in the feeding solution was adjusted to yield a feeding rate of 68 mg h^{-1} mineral nitrogen. The xylan concentration in the feeding solution was now 6 g dm^{-3}, leading to less dilution of the culture broth, while the feeding rate was kept identical to the previous culture. The result showed that biomass grew in a linear manner during the whole fed-batch stage. It can be concluded that this microorganism can develop on a carbohydrate and a mineral nitrogen source. During the first 6 h of the fed-batch stage, xylanase production developed in the same way as in the former culture. The slightly higher level at the 14th hour was a consequence of lower dilution in this fed-batch. It can also be noted that the xylanase synthesis continued after the 14th hour, but an inflection of the graph shows that the productivity decreased. Xylanase synthesis ceased completely after 18.5 h. Besides this, ammonia analysis revealed that mineral nitrogen concentration remained above 100 mg dm^{-3} in the fermenter during the whole fermentation, and reducing sugars were always below 0.1 g dm^{-3} (data not shown). This experiment revealed that xylanase synthesis could be extended for about 4.5 h by supplying mineral nitrogen, with a slight increase of the specific productivity (xylanase activity/biomass) (Table 1).

Since the enzyme synthesis probably needs certain free amino acids or another growth factor, the last culture was supplied with the entire culture medium to avoid any substrate limitation and to explore how long the enzyme synthesis could be maintained. The feeding solution contained 9 g dm^{-3} of soluble xylan and all the other components of the culture medium in the usual proportions. The xylan-feeding rate has been diminished to 1.08 g h^{-1} since there were also peptones present, which represent a supplementary carbon source. Figure 3 shows that biomass grew continuously during the whole fed-batch stage with an average growth rate of 0.06 h^{-1}. The xylanase synthesis was also linear and reached a remarkable level of 20 240 nkat cm^{-3} after 22.25 h of culture time, which corresponds to a productivity of 910 nkat cm^{-3} h^{-1} (Table 1). Reducing sugar concentration remained below 0.1 g dm^{-3} (data not shown). This experiment reveals that the enzyme synthesis phase can be extended up to 15 h with a constant productivity rate by supplying the complete culture medium. Compared with the former fermentation, this strategy gave a gain of 5 h of enzyme production. The specific productivity (xylanase activity/biomass) was almost doubled (Table 1), suggesting that the complete culture medium contains a nutrient element that is essential for enzyme synthesis.

In addition, fermentation gave the same result when the carbon dioxide supply was stopped after 5 h of the batch culture. After this period, the biomass present in the fermenter was able to produce sufficient carbon dioxide itself. In this way, expensive carbon dioxide sparging can be limited for an industrial production process.

Table 1. Comparison of the three fed-batch cultures presented in Section 3.2

Fed-batch	*Time (h)*	*Biomass (g dm^{-3})*	*Xylanase activity (nkat cm^{-3})*	*Xylanase productivity (nkat cm^{-3}h^{-1})*	*Xylanase activity/biomass (nkat g^{-1})*
1	14	1.39	6604	472	4751×10^3
2	18.5	1.59	8973	485	5643×10^3
3	22.25	2.21	20240	910	9158×10^3

4 DISCUSSION

The first two experiments confirmed the double role of xylo-oligosaccharides in xylanase production of this *Bacillus* species; on the one hand they are indispensable for induction of enzyme synthesis, on the other hand their presence in extremely small quantities of $0.1\,g\,dm^{-3}$ xylose equivalents leads to catabolite repression. In conclusion, the most adapted processes that take into account this characteristic are substrate limited fed-batch or continuous cultures. In a fed-batch culture, where biomass developed first on glucose, an undesirable delay of microbial activity was observed at the beginning of feeding with xylo-oligosaccharides, probably due to a change of metabolism. This delay can only be by-passed when the batch stage is also realised on xylan as carbon source. Samain *et al* carried out an identical culture but, in contrast to our results, did not observe this delay: growth and xylanase production started directly with the supply of the xylo-oligosaccharides solution.[7]

The last three fed-batch cultures presented in this paper demonstrated that the supply of ammonia with the carbon source does not enhance significantly the specific productivity, but that the complete culture medium contains an essential factor for enzyme synthesis, as the specific productivity was almost doubled.

The best result reported with this *Bacillus* strain was a fed-batch culture with constant supply of a mixture of glucose, xylo-oligosaccharides and a protein hydrolysate. The enzyme production was maintained for 10 h and reached an activity of $16\,666\,nkat\,cm^{-3}$.[7]

The process proposed in this paper has the advantage of renouncing awkward xylo-oligosaccharides preparation. With the supply of a complete culture medium with xylan as carbon source, the enzyme production phase could be prolonged up to 15 h, leading to an activity of $20\,240\,nkat\,cm^{-3}$. This is one of the highest xylanase concentrations with one of the highest productivities ($910\,nkat\,cm^{-3}h^{-1}$) ever reported.

In the literature, only one further fed-batch culture with a *Clostridium* species has been found. The authors compared several constant feeding rates, and their best result was an activity of $433\,nkat\,cm^{-3}$ with a productivity of $11\,nkat\,cm^{-3}h^{-1}$.[11]

To the best of our knowledge, the highest xylanase production with a *Bacillus* species in a batch culture attained a final concentration of $6600\,nkat\,cm^{-3}$ but with a productivity of $138\,nkat\,cm^{-3}h^{-1}$.[12] Only few fungal cultures have been reported to produce higher xylanase activities in batch cultures of the order of 25 000-38 000 $nkat\,cm^{-3}$ but with a productivity not more than $260\,nkat\,cm^{-3}h^{-1}$.[13-15]

Obviously, fed-batch cultures have been very rarely studied for xylanase production. One might imagine that these processes possess a considerable potential for enhancement of production and productivity, in particular with a careful choice of the feeding strategy, eg fed-batch with a controlled specific growth rate or an exponential feeding rate.

ACKNOWLEDGEMENTS

This work was financed by the programme 'Human Capital and Mobility' of the European Union from which the first author was the recipient of a post-graduate scholarship.

REFERENCES

1 Viikari L, Kantelinen A, Sunquist J and Linko M, Xylanases in bleaching: from an idea to the industry. *FEMS Microbiol Rev* l3:335-350 (1994).

2 Bajpai P, Application of enzymes in the pulp and paper industry. *Biotechnol Prog* l5:147-157 (1999).

3 Coughlan MP and Hazlewood GP, /J-1,4-D-Xylan-degrading enzyme systems: biochemistry, molecular biology and applications. *Biotechnol Appl Biochem* l7:259-289 (1993).

4 Samain E, Debeire P, Debeire-Gosselin M and Touzel JP, Xylanase, xylanase-producing *Bacillus* and applications thereof. Patent WO 92/13942 (1992).

5 Pham PL, Production de xylanases par *Bacillus*. Application au blanchiment sans chlore des pâtes à papier. PhD thesis, INP Toulouse, France (1996).

6 Pham PL, Strehaiano P and Taillandier P, Effect of aeration on xylanase production by *Bacillus* sp I-1018. *Bioprocess Eng* l8:41-43 (1998).

7 Samain E, Debeire P and Touzel JP, High level production of a cellulase-free xylanase in glucose-limited fed batch cultures of a thermophilic *Bacillus* strain. *J Biotechnol* 58:71-78 (1997).

8 Schneider G, Taillandier P and Strehaiano P, Influence of CO_2 on growth and xylanase production of a Bacillus strain, in *Proceedings of the 9th European Congress on Biotechnology*, 11-15 July 1999, Brussels, Belgium (1999).

9 Schneider G, Taillandier P and Strehaiano P, Stoichiometry of diauxic growth of a xylanase producing *Bacillus* strain. *Can J Microbiol* 46 (9):784-789 (2000).

10 Miller GL, Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal Chem* 3l:426-428 (1959).

11 Brodel B, Samain E and Debeire P, Regulation and optimization of xylanase production in *Clostridium thermolacticum*. *Biotechnol Lett* l2 (1):65-70 (1990).

12 Ratto M, Poutanen K and Viikari L, Production of xylanolytic enzymes by an alkali tolerant *Bacillus circulans* strain. *Appl Microbiol Biotechnol* 37:470-473 (1992).

13 Haltrich D and Steiner W, Formation of xylanase by *Schizophyllum commune*: effect of medium components. *Enzyme Microb Technol* l6:229-235 (1994).

14 Purkarthofer H, Sinner M and Steiner W, Cellulase-free xylanase from *Thermomyces languginosus*: optimization of production in submerged and solid-state culture. *Enzyme Microb Technol* l5:677-682 (1993).

15 Bailey MJ, Buchert J and Viikari L, Production of xylanase by *Trichoderma reesei*, in *Proceedings of the second TRICEL symposium on Trichoderma reesei and other Hydrolases, Espoo, Finland 1993*, Ed by Suominen P and Reinikainen T, Foundation for Biotechnical and Industrial Fermentation Research, Vol 8, pp 247-252 (1993).