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Physiological Aspects Involved in Production of Xylanolytic Enzymes by Deep-Sea Hyperthermophilic Archaeon *Pyrodictium abyssi*

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Abstract

Xylanases (EC 3.2.1.8) catalyze the hydrolysis of xylan, the major constituent of hemicellulose. The use of these enzymes could greatly improve the overall economics of processing lignocellulosic materials for the generation of liquid fuels and chemicals. The hyperthermophilic archaeon *Pyrodictium abyssi*, which was originally isolated from marine hot abyssal sites, grows optimally at 97°C and is a prospective source of highly thermostable xylanase. Its endoxylanase was shown to be highly thermostable (over 100 min at 105°C) and active even at 110°C. The growth of the deep-sea archaeon *P. abyssi* was investigated using different culture techniques. Among the carbohydrates used, beech wood xylan, birch wood glucuronoxylan and the arabinoxylan from oats pelt appeared to be good inducers for endoxylanase and β-xylosidase production. The highest production of arabinofuranosidase, however, was detected in the cell extracts after growth on xylose and pyruvate, indicating that the intermediate of the tricarboxylic acid cycle acted as a nonrepressing carbon source for the production of this enzyme. Electron microscopic studies did not show a significant difference in the cell surface (e.g., xylanosomes) when *P. abyssi* cells were grown on different carbohydrates. The main kinetic parameters of the organism have been determined. The cell yield was shown to be very low owing to incomplete substrate utilization, but a very high maximal specific growth rate was determined (μ_{max} = 0.0195) at 90°C and pH 6.0. We also give information on

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the problems that arise during the fermentation of this hyperthermophilic archaeon at elevated temperatures.

Index Entries: Xylanases; *Pyrodictium abyssi*; hyperthermophilic; archaea.

Introduction

Xylan, a 1,4-β-glycoside-linked polymer of D-xylose, is one of the most widespread carbohydrates in nature. The polymer can be catabolized by the synergistic action of several hydrolytic enzymes including endo-xylanases, β-xylosidases, and debranching enzymes. Examples of debranching enzymes are α-L-arabinofuranosidase, α-glucuronidase, and acetyl-xylan-esterase, which liberate the side chains α-L-arabinose, glucuronic acid, and acetate respectively, producing pentoses, which can be further metabolized.

The hyperthermophilic archaeon *Pyrodictium abyssi*, which was originally isolated from marine hot abyssal sites, grows optimally at 97°C *(1)* and is a prospective source of highly thermostable xylanases *(2,3)*. Its endoxylanase was shown to be highly thermostable (over 100 min at 105°C) and active even at 110°C.

Hyperthermophilic microorganisms (those that grow above 90°C and optimum temperature of at least 80°C), which are mostly archaea *(4,5)*, have been investigated for clues to evolutionary processes as well as to uncover biologic strategies underlying life at elevated temperatures. Most of the focus has been on characterization of new isolates and on the mechanisms responsible for thermostability. Although just as important, the physiology of this novel group has been less studied. This is not surprising because hyperthermophiles are generally difficult to culture, in addition to the fact that no generic systems are available for directed analysis of cellular phenomena *(6)*.

The development of cultivation protocols for hyperthermophilic microorganisms presents some interesting problems that are not encountered when working with more conventional organisms growing at mesophilic temperatures. Probably the most significant problem is the relatively little information generated to date on the growth and metabolism of hyperthermophiles. Biochemical and enzymologic research, however, is often limited by the low biomass yields that can be reached for many hyperthermophiles. Consequently, research on the sugar metabolism of hyperthermophiles has been carried out particularly with a few well-culturable species, such as *Sulfolobus* sp., *Thermotoga maritima*, and *Pyrococcus furiosus (7)*.

Along these lines, production of large amounts of biomass presents the unfavorable prospect of very poor volumetric efficiency of fermentors with the additional problem of dealing with the hazards and corrosivity associated with high levels of biologically generated hydrogen sulfide. Thus, difficulties with cultivation of hyperthermophiles represent the key technologic roadblock *(8)*.

The studies reported herein were undertaken to better characterize some parameters involved in the cultivation of the hyperthermophilic crenarcheote *P. abyssi*. The regulation of xylan assimilation of *P. abyssi* and xylanase production were also investigated.

Materials and Methods

Chemicals

All chemicals were of analytical grade and obtained from Merck (Darmstadt, Germany) unless otherwise stated. *p*-Nitrophenylglycosides, oat-spelt xylan, and arabinose were purchased from Fluka (Switzerland); birch wood xylan was from Carl Roth GmbH (Karlsruhe, Germany); beech wood xylan was from Lenzing (Lenzing, Austria); yeast extract was from Gibco (Eggenstein, Germany); Pefabloc and pyruvate were from Boehringer (Mannheim, Germany), and cationized ferritin from was Sigma (München, Germany).

Organism and Growth Conditions

The anaerobic extremely thermophilic archaeon *P. abyssi* (DSM 508) was obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany). The cells were cultivated anaerobically in modified SME medium *(9)* and the pH was adjusted to 5.5 with H_2SO_4. Cultures were grown in 100- and 1000-mL serum bottles at 97°C without shaking (gas phase: H_2/CO_2, 80:20; 0.1–0.2 MPa of overpressure).

Induction Experiments

The induction experiments were carried out in 1000- and 2000-mL serum bottles containing 500 and 1000 mL of basal medium (initial pH 5.5) supplemented with 0.2% (w/v) mono- or polysaccharides. Initial cell concentration in each culture was about 1.0×10^6 cells/mL. Growth in the presence of different substrates was conducted using H_2/CO_2 atmosphere (80:20, 0.1–0.2 MPa of overpressure). The cultures were incubated at 97°C for 48 h.

To localize xylanase activity (cell-associated or cell-free xylanase), sonicated cell pellets and the corresponding concentrated culture supernatant were prepared and examined for relative levels of xylanolytic activity.

Batch Production of Endoxylanases

The basic synthetic medium SME was used and oat-spelt xylan (0.5% [w/v]) or arabinose (0.5% [w/v]) was used as the carbon source. The cultures were grown in 2000-mL serum bottles at 97°C for 48 h and used as inoculum for a 16-L bioreactor (Bioengeneering, Wald, Switzerland) with a working volume of 13 L. At different time intervals, 20-mL samples were taken and enzyme activity, amount of reducing sugars, and pH were monitored. The

initial pH of the medium was 5.5 and was not adjusted during the fermentation. Fermentation was carried out at 90°C, without stirring, with a gas phase of H_2/CO_2 (80:20) and 0.1–0.12 MPa of overpressure.

Enzyme Assays

Xylanase activity was determined by the method of Somogyi *(10)* using birch glucuronoxylan (Carl Roth GmbH) as substrate in universal buffer (0.12 *M*, pH 6.0). The reaction mixture was incubated at 95°C for 15–30 min, the reaction was stopped on ice, and the liberated reducing sugars were assayed. One unit of enzyme activity was defined as the amount of enzyme that released 1 µmol of reducing sugar (xylose as standard)/min under the assay conditions specified. Xylosidase and arabinofuranosidase were assayed using *p*-nitrophenyl-β-D-xylopyranoside and *p*-nitrophenyl-α-L-arabinofuranoside, respectively. One unit of enzyme activity was defined as the amount of enzyme that released 1 µmol of *p*-nitrophenol/min under the assay conditions described. The total amount of reducing sugars was determined colorimetrically at 540 nm by the dinitrosalicylic acid method *(11)*, using xylose as standard. Protein concentration was determined using the method of Bradford *(12)*, with bovine serum albumin as standard.

Scanning Electron Microscopy

To evaluate the presence of xylanosomes, *P. abyssi* cells grown on glucose and xylan as the sole carbon source were examined. Cell samples (10 mL) were centrifuged for 20 min at 12,000*g*, washed, recentrifuged, and resuspended with 1-mL aliquots of 0.15 *M* saline solution. Cells were labeled with cationized ferritin suspension (1 mg/mL), the specimens were dried with a series of graded (20–100%) ethanol solutions, and then they were critical point dried with liquid CO_2. Cells were coated with gold and viewed with a Leitz electron microscope. Controls were also prepared as just described, except that they were not incubated with cationized ferritin.

Prediction of Growth Model Parameters and Statistical Analysis

The kinetic parameters of the Monod, Contois, and Tessier models (Eqs. 1–3) were estimated using a nonlinear method. The calculated responses (cell mass and substrate concentration) were obtained from the numerical solution of the equations set composed by each of the growth models plus the relation between substrate uptake and growth rates (Eq. 4).

$$\frac{dX}{dt} = \frac{\mu_{max} \cdot X \cdot S}{K_s + S} \tag{1}$$

$$\frac{dX}{dt} = \frac{\mu_{max} \cdot X \cdot S}{B \cdot X + S} \tag{2}$$

$$\frac{dX}{dt} = \mu_{max} \cdot \left[1 - e^{(-S/K_S')}\right] \tag{3}$$

$$\frac{dS}{dt} = -\frac{1}{Y_{X/S}} \cdot \frac{dX}{dt} \tag{4}$$

For statistical analysis, response variables were assumed in a linearized context, in such a way that the confidence limits for correct responses are given by (Eq. 5):

$$\boldsymbol{y} - t_{v,1-\alpha/2}\left\{S_R^2 \boldsymbol{diag}\left[\mathbf{J}(\mathbf{J}^t\mathbf{J})^{-1}\mathbf{J}^t\right]\right\}^{1/2} < \eta < \boldsymbol{y} + t_{v,1-\alpha/2}\left\{S_R^2 \boldsymbol{diag}\left[\mathbf{J}(\mathbf{J}^t\mathbf{J})^{-1}\mathbf{J}^t\right]\right\}^{1/2} \tag{5}$$

The standard deviations of the parameters were the square roots of the variances drawn from the main diagonal of the covariance matrix of estimated parameters (Eq. 6):

$$\mathbf{Cov}(\hat{\beta}) = \sigma_\varepsilon^2 \cdot (\mathbf{J}^t \cdot \mathbf{J})^{-1} \tag{6}$$

in which the coherent and unbiased estimator used for the error variance (σ_ε^2) was

$$S_R^2 = \frac{\sum_i^n (y_i - \hat{y}_i)^2}{n - p} \tag{7}$$

All these calculations were coded in MATLAB 4.2 c.1 (Mathworks).

Results and Discussion

Effect of S^0 on Cell Growth

P. abyssi grew well in the medium containing S^0 as a terminal electron acceptor with proteinaceous material (yeast extract) as the sole carbon source, as previously reported *(1)*. When the concentration of yeast extract was increased to 0.25%, the final cell density was at least one order of magnitude higher. As shown in Table 1, growth was observed also in the absence of S^0. When 0.5% xylan was added to the medium containing 0.05% yeast extract, cell concentration increased from 4.0×10^7 to 1.18×10^8. Growth on insoluble beech xylan was not affected by the absence of sulfur in the medium (Table 1).

P. abyssi showed the best growth rate, with a doubling time of 2.5 h, on medium containing 0.5% yeast extract in the absence of S^0. As shown in Table 2, xylanase activity was not present when growth was performed with a medium without xylan. The addition of xylan to the minimal medium (0.05% yeast extract) was accompanied by the formation of endoxylanase

Table 1
Effect of S^0 and Carbon Source on Growth of *P. abyssi*[a]

Medium[b]	Substrate concentration	Incubation time (h)	Cells/mL[c]
SME[d]	0.05% YE, 0.05% S^0	40	2.12×10^7
Minimal[e]	0.05% YE	40	4.00×10^7
YE	0.25% YE	24	3.20×10^8
Tryptone	0.25% Try	24	3.10×10^8
Beech xylan	0.05% YE, 0.5% Xyl	40	1.18×10^8

[a]YE, yeast extract; S^0, sulfur; Try, tryptone; Xyl, insoluble xylan (Lenzing A.G.).
[b]Cultures were grown in 100- and 500-mL serum bottles at 97°C without shaking (gas phase H_2/CO_2, 80:20; 0.1-MPa overpressure).
[c]Cell number was estimated in a Neubauer counting chamber; a starting inoculum of 1.5×10^6 was used.
[d]SME medium *(9)*.
[e]SME medium without sulfur.

Table 2
Effect of Carbon Source
on Xylanase Production, Growth Rate, and Doubling Time of *P. abyssi*

Fermentation[a]	Initial cell number (cell/mL)	Endoxylanase[b] (U/L)	Growth rate (h^{-1})	Doubling time[c] (h)
0.5% YE	1.12×10^7	0 (14 h)	0.41	2.5
0.05% YE + 0.2% beech xylan	1.53×10^7	0.27 (16 h)	0.28	3.5
0.05% YE + 0.5% xylose	1.37×10^7	0.29 (19 h)	0.21	4.7
0.05% YE + 0.5% arabinose	0.12×10^7	0.14 (93 h)	0.12	8.1

[a]YE, yeast extract. The 16-L fermentor was operated without stirring with a gas phase of H_2/CO_2 (80:20) and 0.1–0.12 MPa of overpressure at 93°C.
[b]Fermentation was stopped at the stationary phase and xylanase activity was measured in the cell extract and concentrated supernatant. Enzyme activity is expressed as described in Materials and Methods.
[c]Doubling time was calculated from the slopes of the growth curve (not shown).

(Table 2). Poor growth was observed when xylan was replaced with arabinose.

The obligate chemoorganotrophic *P. abyssi* grows organotrophically with complex substrates such as xylan, while many extremely thermophilic archaea grow with sulfur and H_2 as the sole energy substrates *(13)*, indicating that *P. abyssi* does not necessarily have to reduce sulfur. Fermentation of organic substrates in the absence of elemental sulfur has been reported for several extremely thermophilic sulfur reducers *(5)*. Usually, the final cell densities of these bacteria in batch cultures are low *(14)*, from 10^6 to

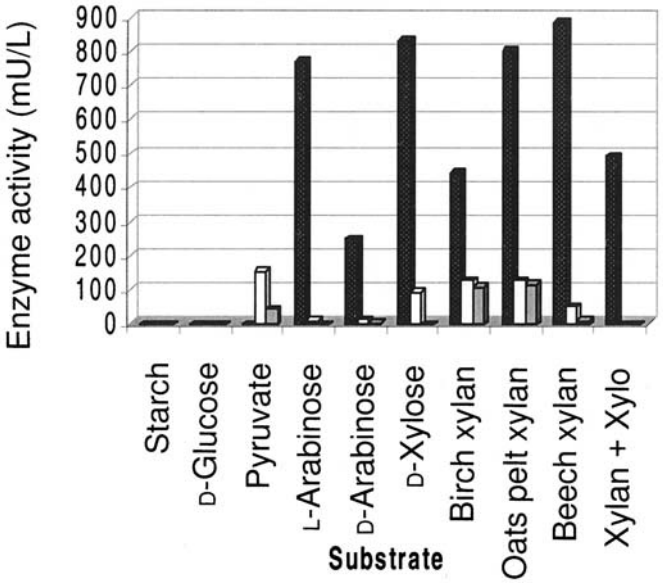

Fig. 1. Production of endoxylanase (■), arabinofuranosidase (□) and betaxylosidase (▨) by *P. abyssi*. Growth on the different substrates was determined in the presence of H_2/CO_2 atmosphere (80:20, 0.1–0.2 MPa of overpressure). The initial cell concentration was about 1.0×10^6 cells/mL and the cultures were incubated at 97°C for 48 h. Sonicated cell pellets and the corresponding clarified culture supernatant were prepared and examined for activity. Enzyme activity is expressed as described in Materials and Methods.

10^7 cells/mL, which was also observed for *P. abyssi*. Higher cell densities were observed for *P. abyssi*, when the organism was cultivated on high concentrations of yeast extract or starch (>10^8 cells/mL).

Influence of Various Carbon Sources on Xylanase Activity

Figure 1 shows total (extracellular and cell-bound) endoxylanase, arabinofuranosidase, and β-xylosidase production in the extreme thermophilic archeon *P. abyssi* after 48 h of growth with the various carbon sources tested. Enzyme production in *P. abyssi* seems to be inducible in the presence of various xylans and xylose. The xylanolytic enzymes endoxylanase, arabinofuranosidase, and β-xylosidase were not synthesized in the presence of starch or glucose (Fig. 1).

P. abyssi degrades xylan by the coordinate action of a complex of hydrolyzing enzymes, which act together in a concerted manner to effectively degrade the substrate. The findings in this study suggest that xylan-degrading enzymes, including endoxylanase, arabinofuranosidase, and β-xylosidase, were predominantly associated with the cell during growth on xylan, except under conditions of xylan limitation in which endoxylanase became predominantly extracellular. Immunologic and electron microscopic studies with monoclonal antibodies raised against endo-

xylanase will be necessary to provide conclusive evidence for the cellular location of the xylanolytic enzymes.

Although starch was the best carbon source for growth of the organism, it was a poor substrate for xylanase production. The xylanolytic enzymes seem to be regulated by induction and repression. Similar results were observed for the mesophilic actinomycete *Streptomyces avermitilis (15)*, thermophilic actinomycete *Thermonospora curvata (16)*, and thermophilic fungus *Thermomyces lanuginosus (17)*. Synthesis of the xylanolytic enzymes in *P. abyssi* is regulated by the presence of substrates containing pentose. The levels of xylanolytic enzymes in crude culture supernatants varied greatly in response to the carbon source used for growth. Since the secreted enzyme was highly stable, showing activity even after 100 min of incubation at 105°C, enzyme synthesis but not enzyme turnover is likely to play a major role in mediating the level of extracellular activity.

The regulatory systems for biosynthesis of xylanase studied so far are mainly from mesophilic organisms, including the filamentous fungi of the genera *Aspergillus* and *Trichoderma*. Several thermophilic organisms have been reported to produce thermostable xylanases, but little information is available concerning the biosynthetic regulation of xylanases in thermophilic organisms. The regulatory mechanisms involved in xylan degradation by thermophilic anaerobic bacteria are still not well understood. In previous studies on the regulation of xylanase synthesis, two mechanisms have been proposed. First, in many xylan-degrading microorganisms, the xylanolytic enzymes appear to be inducible *(18,19)*. The synthesis of xylanolytic enzymes can be inhibited by the presence of glucose or some other sugars in the growth medium. The synthesis is then regulated by an induction-repression mechanism. Because polysaccharides cannot enter the cells, a soluble oligosaccharide such as xylobiose or xylotriose is considered to act as a direct inducer of xylanase synthesis. The oligosaccharides are formed by the hydrolysis of xylan in the medium by low amounts of enzyme that are produced constitutively. Induction is also possible with some synthetic alkyl-, aryl-, and methyl-β-D-xylosides, as well as positional isomers of xylobiose such as 1,2-β-xylobiose *(18,19)*. Second, in other xylan-degrading bacteria, the xylanase synthesis is considered to be constitutive but repressed by conditions favoring optimal growth. The xylanase synthesis is subject to control by growth rate–dependent repression by readily metabolized carbon sources *(18)*.

For the anaerobic bacterium *Bacteroides xylanolyticus*, pyruvate appeared to be a stronger inducer *(18)*. Extracellular xylanase production in the anaerobic thermophilic *Dictyoglomus* sp. B1 was achieved at high levels using insoluble beech wood xylan *(20)*. On the other hand, the β-xylosidase from *Trichoderma reesei* was induced by xylose and beech wood xylan, while L-arabinose induced enzyme production at a very low level *(19)*. By contrast, the thermophilic *T. lanuginosus* has shown low constitutive levels of enzymes, endoxylanase, and β-xylosidase, using a variety of substrates, including birch xylan, maltose, xylose, and bagasse *(17)*. L-Sor-

bose induced xylanase activity as well as cellulase in *T. reesei*. It induced a higher level of xylanase activity than sophorose and xylose did *(21)*. The arabinofuranosidase from the mesophilic anaerobic bacterium *B. xylanolyticus* X5-1 *(18)* was induced by L-arabinose and xylose but was poorly induced by oat-spelt xylan.

In the case of *P. abyssi*, the highest levels of endoxylanase were obtained with beech wood and oat-spelt xylan. The highest levels of β-xylosidase production were observed with the naturally occurring xylan, oat spelt, and birchwood xylan. The highest production of arabinofuranosidase, however, was detected in the cell extracts after growth on xylose and pyruvate, indicating that the intermediate of the tricarboxylic acid cycle acted as a nonrepressing carbon source for the production of this enzyme.

Although other bacteria are able to utilize L-arabinose anaerobically *(18)* or even aerobically *(22)*, bacteria do not commonly metabolize D-arabinose. Interestingly, the hyperthermophilic archaeon *P. abyssi* is able to induce xylanase production with both enantiomeric forms. Although xylan is the dominant pentosan and glucomannan the dominant hexosan, the level of arabinan is significant in some biomass materials *(23)*. The ability of microorganisms to ferment L-arabinose in the context of conversion of hemicellulosic sugars is extremely important. In the fermentation of pentoses two pathways are possible: direct breakdown of xylulose 5-phosphate by the enzyme phosphoketolase or the conversion of xylulose 5-phosphate to hexose phosphate prior to metabolism in the pentose phosphate pathway. The pentose phosphate pathway is the most common mechanism of pentose catabolism in anaerobic bacteria *(24,25)*.

The availability of xylan and other plant-derived polysaccharides in the marine hot abyssal environments is likely and may transiently provide resident cells with a growth advantage. Regulation of xylanase syntheses in the hyperthermophile *P. abyssi* provides an energy-efficient means for the utilization of such polysaccharides.

The specific activity of xylanases produced by *P. abyssi* in batch culture was lower than 0.1 mU/L for β-xylosidase and arabinofuranosidase. The specific activity of endoxylanase on xylan was 0.24–0.41 mU/mg and on arabinose 0.64 mU/mg (Table 3). Usually, the specific activities determined for crude xylanase preparations from different xylan-degrading organisms grown in batch cultures are relatively low, usually ranging from 0.06 to 50 U/mg *(26,27)*. However, higher specific activities up to 200 U/mg were already described for a thermostable xylanase from *Clostridium stercorarium (28)* and for the xylanase from *Dictyoglomus* sp. *(20)*. The specific activity measured for the xylanase of *P. abyssi* was very low, but comparable with those obtained by Canganella et al. *(13)* for amylases and pullulanases from archaea. The low specific activities could be owing to the yeast extract concentration in the medium that was required to obtain acceptable growth yield in the absence of elemental sulfur.

Table 3
Effect of Growth Substrates on Specific Activities of Xylanolytic Enzymes of *P. abyssi*[a]

Substrate (0.2%)	Protein (mg/L)[b]	Endoxylanase[c]		Arabinofuranosidase[c]		β-Xylosidase[c]	
		(mU/L)	Specific activity (mU/mg)	(mU/L)	Specific activity (mU/mg)	(mU/L)	Specific activity (mU/mg)
L-Arabinose	1254	810	0.64	10	0.008	0	0
D-Arabinose	1068	251	0.23	18	0.016	>10	0
D-Xylose	1710	308	0.18	177	0.10	0	0
Birch xylan	1822	442	0.24	46	0.025	92	0.050
Beech xylan	2364	984	0.41	67	0.028	>10	0
Oat-spelt xylan	2730	886	0.32	68	0.025	12	0.004

[a]Growth on the different substrates was determined in the presence of H_2/CO_2 atmosphere (80:20, 0.1–0.2 MPa of overpressure). The cultures were incubated at 97°C for 48 h.
[b]Protein was measured by the method of Bradford *(12)* and represents the total protein (supernatant and cell extract).
[c]Enzyme activity is expressed as described in Materials and Methods.

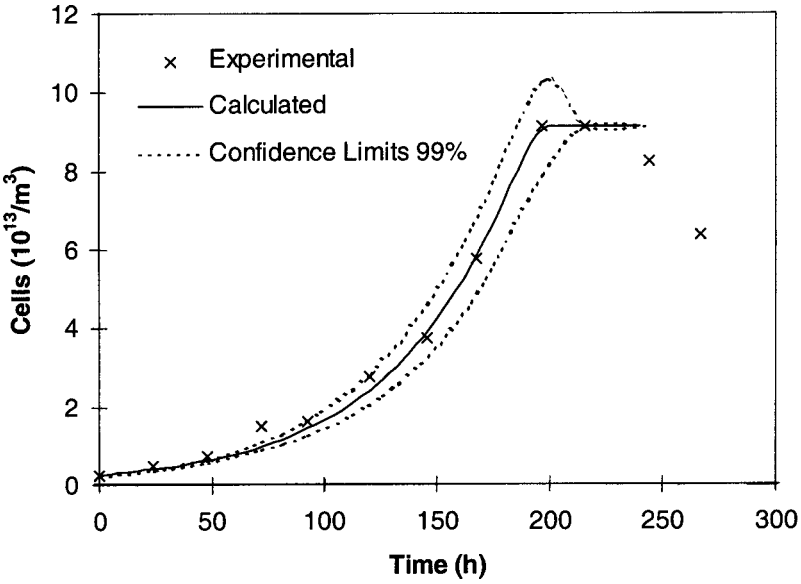

Fig. 2. Growth model parameter prediction of *P. abyssi* during growth on 0.5% xylan. The medium contained 0.5% (w/v) oat-spelt xylan. Fermentation was carried out in the 16 L fermentor at 90°C, without stirring, with a gas phase of H_2/CO_2 (80:20) and 0.1–0.12 MPa of overpressure. Total extracts were evaluated for xylanase activity and reducing sugars as described in Materials and Methods.

Cultivation of P. abyssi *in Batch Culture*

To investigate xylanase production in batch experiments, L-arabinose and oat-spelt xylan were used as substrates. During growth on oat-spelt xylan, the maximum xylanase activity reached 390 mU/L after 120 h. After this point the endoxylanase activity decreased and was 120 mU/L after 264 h. From the experimental data (Fig. 2), the kinetic parameters of Monod (Eq. 5), Contois (Eq. 6), and Tessier (Eq. 7) models were estimated (Table 4). In Fig. 2 the solid lines show estimated responses for the Contois model and the dotted lines delimit the confidence region for the correct responses (Eq. 14) at the 99% level. The calculated responses of these models with their estimated parameters were only slightly different and were represented by the same determination coefficient (R^2) values (Table 4).

During cultivation on arabinose, endoxylanase activity was lower, reaching 142 mU/L after 120 h and decreasing gradually to 23 mU/L after an additional 100 h. The total reducing sugar concentration at the end of the fermentation was 22 m*M*, indicating that sugars were consumed very slowly (data not shown). The turbidity caused by the turbid xylan-containing medium prevented the measurement of cell growth by absorbance. In experiments with arabinose, the formation of Maillard products did not allow the determination of growth using turbidimetric methods.

The adequacy of the Monod equation form (Eq. 5) to describe substrate-limited growth is normally related to low cell population *(29)*. On the

Table 4
Growth Parameter Prediction by Different Models

	Monod		Contois		Tessier		—
Parameter	μ_{max} (h^{-1})	K_s (kg/m^3)	μ_{max} (h^{-1})	B (kg S/10^{13} cells)	μ_{max} (h^{-1})	K_s' (kg/m^3)	μ_x (h^{-1})
Estimate	0.0195	0.1532	0.0189	0.0163	0.0188	0.0803	0.0174
Standard deviation	0.012	0.29	9.3×10^{-3}	0.029	7.7×10^{-3}	23	—
R^2	0.9898		0.9898		0.9898		—

[a]K_s, Monod saturation constant; K_s', Tessier apparent saturation constant; μ_{max}, maximum specific uptake rate.

other hand, the Tessier form (Eq. 7) seems to present similar behavior since its apparent Michaelis constant (K_s') can be directly related to the true one by means of $K_s = \ln(2) \cdot K_s'$. According to this model, it can be shown that the specific growth rate is half of the maximum when $S = \ln(2) \cdot K_s'$.

The Contois model (Eq. 6) usually appears more appropriate for high-density cell cultures *(29)* since it has a pseudo–Michaelis constant proportional to biomass concentration. The standard deviations of estimated parameters (Table 4), obtained from square roots of the main diagonal of Eq. 13, showed smaller values for parameters of the Contois model than the other ones. This implies smaller variability for Contois estimates than for the Monod and Tessier models. The physical significance of its parameters and its acceptability for high cell density cultures pointed to the choice of the Contois model as the most indicated to predict the growth of *P. abyssi* on xylan.

The cultivation of *P. abyssi* in a fermentor without stirring was successful, although agitation is usually necessary to maintain the medium homogeneity in order to avoid the formation of large metabolic inactive pellets. Most hyperthermophiles investigated so far grow optimally at an agitation speed of 2000 rpm *(8)*. However, shearing forces may also disrupt fragile microbial cell networks, such as the network of *P. abyssi*, and can have a marked influence on xylanase production. *P. abyssi* cells are reminiscent of fungal mycelia *(1)*, and disruption of the network causes cessation of growth with consequent hindrance of xylanase production. This is the first report on fermentation experiments to improve production and secretion of xylanases in archaea.

Presence of Xylanosomes

The presence of xylanosomes on the cell surface of *P. abyssi* was investigated after growth on xylan and glucose. Cationized ferritin-labeled cells grown on glucose were compared with those grown on xylan by scanning electron microscopy. No xylanosomes were observed under the conditions tested (data not shown).

Many anaerobic cellulolytic bacteria posses high molecular weight, multisubunit cellulases that are often associated with the cell surface or sedimentable membranous fragments *(30,31)*. In *Thermoanaerobacter* B6A, the finding of cell-bound endoxylanase activity, a strong affinity of the cells for the substrate, and the presence of cell-surface protuberances suggests the presence of xylanosomes, a structure analogous to the cellulosome *(32)*. However, such structures were not detected in *P. abyssi*. The absence of xylanosomes in this archeon suggests that different strategies could be employed to bind the enzyme to the cell surface, and it is possible that the enzymes from *P. abyssi* are associated with the network of tubules.

Although there are many reports focusing on the microbial degradation of xylan by xylanolytic enzymes, xylanase activity recently has been found also in another archaeon, *Thermococcus zilligii*, which grows optimally at 75°C *(33)*. We were able to show that the hyperthermophilic

P. abyssi can produce xylanolytic enzymes consisting of endoxylanase, arabinofuranosidase, and β-xylosidase, and we have demonstrated that this system is inducible by different carbon sources. The regulation of endoxylanase production appears to be independent of cell growth rate. The synthetic production medium selected for this study with oat-spelt xylan as the carbon source seems to be a good medium for xylanase production. The optimization of the medium composition for a desired enzyme activity profile may provide a way for customized enzyme cocktail production for special purposes such as that required in pulp and paper applications.

Nomenclature

B = Contois apparent saturation constant
$\boldsymbol{diag}(\mathbf{M})$ = vector extracted from main diagonal of generic matrix $\mathbf{M}$
$\mathbf{J}$ = Jacobian matrix of estimated parameters
K_s = Monod saturation constant
K_s' = tessier apparent saturation constant
n = number of responses
p = number of parameters
S = substrate concentration
S_R^2 = estimate of error variance
t = time
$t_{\nu,1-\alpha/2}$ = t-Student abscissa for ν degrees of freedom at the significance level $\alpha/2$.
X = cell mass concentration
y = experimental response variable (cell mass or substrate concentration)
$\hat{y}$ = estimated response variable
$\boldsymbol{y}$ = estimated responses vector
$Y_{X/S}$ = cell mass yield factor
$\hat{\beta}$ = estimated parameters vector
η = correct responses vector
μ_{max} = maximum specific uptake rate
σ_ε^2 = error variance

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