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ORIGINAL PAPER

Effect of cultivation pH and agitation rate on growth and xylanase production by *Aspergillus oryzae* in spent sulphite liquor

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Abstract The effects of cultivation pH and agitation rate on growth and extracellular xylanase production by *Aspergillus oryzae* NRRL 3485 were investigated in bioreactor cultures using spent sulphite liquor (SSL) and oats spelts xylan as respective carbon substrates. Xylanase production by this fungus was greatly affected by the culture pH, with pH 7.5 resulting in a high extracellular xylanase activity in the SSL-based medium as well as in a complex medium with xylan as carbon substrate. This effect, therefore, was not solely due to growth inhibition at the lower pH values by the acetic acid in the SSL. The xylanase activity in the SSL medium peaked at 199 U ml^{-1} at pH 7.5 with a corresponding maximum specific growth rate of 0.39 h^{-1}. By contrast, the maximum extracellular β-xylosidase activity pf 0.36 U ml^{-1} was recorded at pH 4.0. Three low molecular weight xylanase isozymes were secreted at all pH values within the range of pH 4–8, whereas cellulase activity on both carbon substrates was negligible. Impeller tip velocities within the range of 1.56–3.12 m s^{-1} had no marked effect, either on the xylanase activity, or on the maximum volumetric rate of xylanase production. These results also demonstrated that SSL constituted a suitable carbon feedstock as well as inducer for xylanase production in aerobic submerged culture by this strain of *A. oryzae*.

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Introduction

Xylanolytic enzymes constitute a commercially important class of enzymes. Cellulase-free xylanases have a wide range of potential biotechnological applications in the pulp and paper [12, 23], food and beverage [19] and agricultural industries [39]. The efficient production of fungal xylanases is dependent on a number of cultivation parameters that include the nature of the inducing substrate, culture medium composition, temperature, pH, dissolved oxygen tension and shear stress in submerged culture. It is, therefore, necessary to quantify the effects of these parameters to optimise the activity, rate and yield of xylanase production. Some of these effects are species and strain-specific, thereby often rendering generalisations invalid.

The cultivation pH has been shown to be of particular importance in respect of xylanase production by various microorganisms [5, 16, 29, 31]. For example, whereas with *Aspergillus fischeri* fxn1 the maximum xylanase activity was obtained between pH 6 and 10 [10], the optimal pH for xylanase production by strains of *A. fumigatus* was pH 5 [2] or less [7]. Furthermore, the secretion of fungal xylanases is generally associated with extracellular cellulase activity [34]. However, the selective production of xylanase is possible, as was demonstrated with *Trichoderma reesei* where pH 4 favoured cellulase production, whereas cultivation at pH 6–7 favoured xylanase production [5].

In submerged culture, xylanase production by filamentous fungi may also be affected by shear stress, which is related to the agitation rate. The high viscosities and non-

Newtonian behaviour of culture broths of filamentous fungi often necessitate the use of high agitation rates to provide adequate mixing and oxygen transfer. However, mycelial damage due to high shear stress limits the practicable range of stirrer speed and consequently the volumetric biomass and enzyme productivity of the culture. Several papers reported on the effects of agitation rate in combination with the aeration rate and dissolved oxygen tension [21, 28, 33, 35].

Previously we reported on endo-β-xylanase production by seven fungal strains in shake flask cultures with spent sulphite liquor (SSL), a wastewater of the pulp and paper industry, as carbon feedstock. The enzyme characteristics and the efficacy of the xylanases in the biobleaching of *Eucalyptus* pulp were determined [11]. The above shake flask-screening experiments revealed a strain of *Aspergillus oryzae* that was a promising candidate for further investigation. In this paper we report, in more detail, on xylanase production in bioreactor batch culture by this strain of *A. oryzae* to determine the effect of the pH on the culture parameters, as well as on the influence of shear stress, independent of the dissolved oxygen tension. The carbon substrates were SSL as well as oats spelts xylan, a known inducer of xylanase activity in fungi, for comparison.

Materials and methods

Microorganism

The fungal strain used in this study was *Aspergillus oryzae* NRRL 3485, maintained on Sabouraud-dextrose agar slants (Biolab Diagnostics Ltd., South Africa) and stored at 4°C with subculturing at 12-week intervals.

Effect of cultivation pH

Batch cultivations were conducted at 30°C for 60 or 120 h in a 15-l Biostat C bioreactor (B. Braun Biotech International, Germany) with a working volume of 8.5 l. The vessel was equipped with four baffles and three Rushton disc turbine impellers as well as one propeller-type impeller, each measuring 74.4 mm in diameter and fitted equidistantly on the stirrer shaft with the propeller at the top. The dissolved oxygen tension (DOT) in the culture was monitored with a polarographic electrode (Mettler Toledo, UK) and maintained at the desired setpoint by automatic cascade control of the stirrer speed and air flow rate in the range of 0.05–8.0 std l min^{-1}. The pH was controlled by automatic titration with 5 N KOH or 5 N H_2SO_4. A 500-ml mycelial inoculum was added after in situ sterilisation of 8.0 l medium at 121°C for 15 min, giving an inital biomass concentration (dry wt) of 0.3 ($\pm$0.04) g l^{-1}. The inoculum was prepared by transferring 5 ml of a fungal spore suspension with a count of ca. 10^7 spores ml^{-1}, washed from a 5-day-old Sabouraud dextrose agar plate culture with 10 ml of a 0.05-M KH_2PO_4 solution containing 0.1% Tween 80, to each of five 500-ml Erlenmeyer flasks containing 100 ml of a xylan-based medium. These were subsequently incubated at 210 rpm on an orbital shaker at 30°C for 48 h.

The composition of the spent sulphite liquor (SSL) that served as main carbon source is described elsewhere [11]. It was supplied in a concentrated form (designated SSLc) and contained acetic acid that originated from the acetyl groups of the xylan. Prior to use, the SSLc was diluted 20-fold with distilled water, hereafter designated SSL, which had an acetic acid content of just over 1 g l^{-1}. The culture medium constituents added to the SSL were (in g l^{-1}): citric acid, 0.25; $(NH_4)_2SO_4$, 5; K_2HPO_4, 5; $MgSO_4.7H_2O$, 0.5; $CaCl_2.2H_2O$, 0.02; yeast extract, 10 as well as 1 ml of a trace elements solution described elsewhere [15] and 1 ml Dow Corning 1520 Silicone antifoam (BDH Laboratory Supplies, England). For comparative purposes, oats spelts xylan (Sigma Chemical Co., USA) was used as alternative carbon substrate at a concentration of 10 g l^{-1} in a complex medium containing the above constituents (excluding the SSL) made up in distilled water.

Effect of agitation rate

The effect of the agitation rate on xylanase production was investigated in the SSL-based medium at pH 7.5, as above, but for these experiments the SSL was diluted 40-fold. The DOT was controlled at 25% of saturation while maintaining a constant stirrer speed of 400, 500, 600 or 800 rpm, respectively, that corresponded to impeller tip velocities of 1.56, 1.95, 2.34, and 3.12 m s^{-1}, calculated as Πnd_i where n is the stirrer speed and d_i the impeller diameter [26].

Analytical methods

Xylanase activity in the culture supernatants was determined using the dinitrosalicylic acid (DNS) assay method with birchwood xylan (Sigma) as substrate [4]. Cellulase activity was determined using the filter paper assay method [17] and β-xylosidase activity as described elsewhere [25]. Protease activity was determined by incubating a 0.15-ml sample with 0.25 ml of a 2% azocasein (Sigma) substrate for 30 min at 30°C, followed by the addition of 1.2 ml of a 10% trichloroacetic acid solution to stop the reaction. After centrifugation of these samples at 15,000 rpm for 5 min, 1.2-ml aliquots were transferred into tubes containing

1.4 ml of a 1-M NaOH solution and the absorbance determined at 440 nm with a DU 7500 spectrophotometer (Beckman Instruments, USA). A unit of protease activity was defined as the amount of enzyme required to produce an absorbance change of 1.0 in a 1-cm cuvette.

The fungal biomass concentration was gravimetrically determined by filtration of duplicate 10-ml culture samples using GF 50 glass microfibre filters (Schleicher and Schuell, Germany), washing twice with equal volumes of distilled water and drying at 105°C to constant weight. In the presence of particulate xylan in the xylan-based medium, the fungal biomass was estimated from a standard curve of biomass versus protein concentration, using the biuret protein assay [20]. The sugar composition of the SSL and culture supernatants was determined with a Dionex 4000i HPLC (Dionex Corp., USA) and acetic acid with a Hewlett-Packard 5710A gas chromatograph (Hewlett-Packard, USA) as described elsewhere [11].

SDS-PAGE and zymogram analysis

The supernatants of centrifuged *A. oryzae* cultures grown at different pH values in the SSL-based medium were lyophilised and the extracellular proteins separated by SDS-PAGE. Zymograms were performed on protein samples subjected to electrophoresis as above, but with the gel containing 0.1% Remazol Brilliant Blue R-D-xylan (RBB xylan, Sigma), using broad range molecular weight protein standards (Bio-Rad laboratories, USA) as markers as described elsewhere [11].

Results

Effect of culture pH on growth parameters and enzyme production

The growth parameters of *A. oryzae* with SSL as carbon substrate at different culture pH values are shown in Fig. 1. The maximum volumetric rate of xylanase production, xylanase activity as well as the maximum specific growth rate (μ_{max}) reached maximum values of 13.1 U ml^{-1} h^{-1}, 199 U ml^{-1} and 0.39 h^{-1}, respectively, at pH 7.5. The biomass concentration, however, decreased steadily with an increase in culture pH. As a result of this decrease in biomass concentration concomitant with the increase in xylanase activity with increasing pH value, the specific xylanase yield at pH 4 increased fivefold to 35,000 U g biomass^{-1} at pH 7.5. The highest extracellular β-xylosidase activity of 0.36 U ml^{-1} was recorded at pH 4.0 and decreased with increasing pH to 0.18 U ml^{-1} at pH 7.5. The extracellular protease activity reached its highest

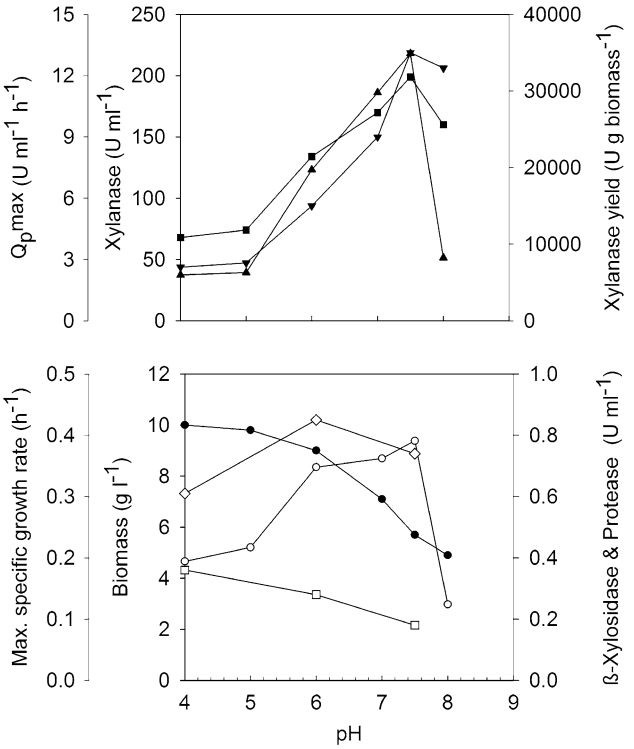

Fig. 1 Growth parameters of *A. oryzae* NRRL 3485 with SSL as carbon substrate as a function of the cultivation pH. Symbols: xylanase activity (*filled square*), maximum volumetric rate of xylanase production, Q_p^{max}(*filled triangle*), specific xylanase yield (*filled inverted triangle*), biomass (*filled circle*), maximum specific growth rate (*open circle*), β-xylosidase activity (*open diamond*), protease activity (*open square*)

value of 0.85 U ml^{-1} at pH 6.0, whereas cellulase production was negligible, namely below 0.07 U ml^{-1}, at all cultivation pH values.

Typical cultivation profiles of *A. oryzae* in the SSL-based medium at pH 4.0 and 7.5 are shown in Fig. 2 for comparison. At pH 4.0, growth and enzyme production were considerably slower than at pH 7.5 (note that the time scales in Fig. 2 differ). At both pH values, rapid xylanase production commenced only after the total sugar concentration in the culture had decreased to a low level. A prolonged growth lag phase was observed at a cultivation pH of 4.0, lasting up to 51 h, during which time the acetic acid concentration in the culture had decreased to a minimal level, and extracellular xylanase activity was detected only after 65 h of cultivation.

Without pH control, the pH of the SSL-based medium increased from an initial value of 5.7–pH 8.4 at the end of the cultivation. Under these conditions the μ_{max} value was 0.356 h^{-1}, which was similar to the value recorded at a controlled pH of 7.0. However, the maximum volumetric rate of xylanase production as well as the specific xylanase yield were sub-optimal and similar to the values obtained at pH 6.0, namely 5.79 U ml^{-1} h^{-1} and 11,800 U g^{-1},

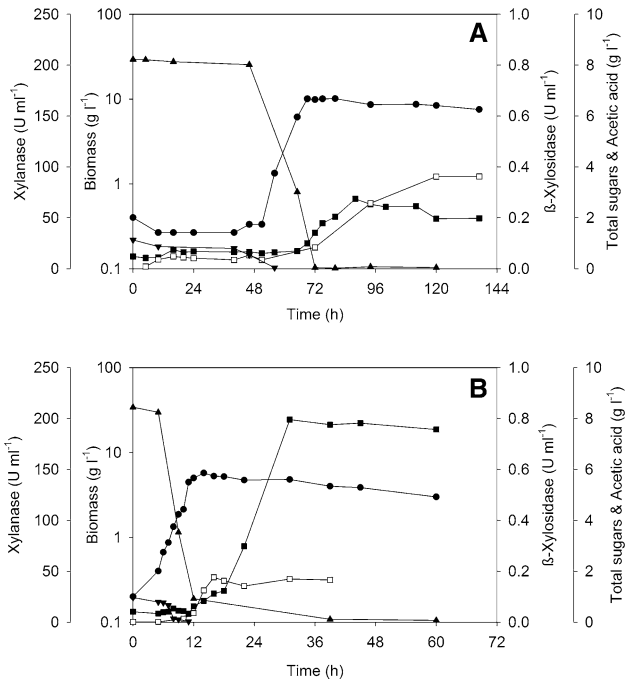

Fig. 2 Typical cultivation profiles of *A. oryzae* NRRL 3485 with SSL as carbon substrate at pH 4.0 (**a**) and pH 7.5 (**b**). Symbols: biomass (*filled circle*), xylanase activity (*filled square*), *β*-xylosidase activity (*open square*), total sugars (*filled triangle*), acetic acid (*filled inverted triangle*)

respectively. The xylanase activity of 153 U ml^{-1} was markedly lower than at a controlled pH of 7.5.

Table 1 shows the growth parameters of *A oryzae* with oats spelts xylan as carbon substrate at different pH values. As found when using the SSL as carbon feedstock, the xylanase yield and especially the volumetric rate of xylanase production increased markedly with an increase in pH from 4.0 to 7.5, whereas the xylanase activity reached maximal values at pH 6 and 7. A change in pH had little effect on the final biomass concentration. These values were higher than when spent sulphite liquor served as carbon feedstock, whereas the maximum specific growth rate was similar. A higher μ_{max} value of 0.187 h^{-1} was obtained on xylan without pH control than in cultures with pH control, but the maximum volumetric rate of xylanase production and the xylanase yield were much lower than at pH 7.5. As with SSL as carbon source, the extracellular ß-xylosidase, cellulase and protease activities obtained on xylan were low.

Typical cultivation profiles of *A. oryzae* with xylan as carbon substrate at pH 4.0 and 7.5 are shown in Fig. 3. The xylanase activities followed a trend similar to those obtained in the SSL-based medium, with the highest xylanase activity of 289 U ml^{-1} obtained at pH 7.5 and a markedly lower activity of 187 U ml^{-1} at pH 4.0 (Fig. 3, Table 1). The cultivation profile at pH 4.0 with xylan as carbon substrate did not exhibit any prolonged growth lag phase and xylanase production began much earlier (Fig. 3a) than was the case with the SSL-based medium (Fig. 2a). *β*-Xylosidase activities were again higher at pH 4.0 (0.49 U ml^{-1}) than at pH 7.5 (0.25 U ml^{-1}) and were also higher than the values recorded in the SSL-based medium. Cellulase activity was negligible (Table 1), as was also found in the SSL-based medium. The highest protease activity of 1.24 U ml^{-1} was recorded at a culture pH of 7.5.

SDS-PAGE and zymograms

A zymogram of *A. oryzae* revealed three xylanase isozymes and all three were produced in the SSL-based medium at each of the cultivation pH values of 4.0, 6.0, 7.5 and 8.0 (not shown). The molecular weights of these xylanase isozymes were 32, 22 and 19 kDa, respectively. A

Table 1 Growth parameters (mean values of duplicate experiments with standard deviation of the mean) of *A. oryzae* NRRL 3485 with xylan as carbon substrate in bioreactor cultures at 30°C and different cultivation pH values

Parameter	Cultivation pH			
	4.0	6.0	7.5	No pH control
Xylanase (U ml^{-1})	187 (±10)	286 (±9)	289 (±17)	153 (±20)
Biomass (g l^{-1})	6.2 (±1.3)	6.7 (±0.9)	6.1 (±0.3)	6.0 (±0.8)
Cellulase (FPUs)	0.06 (±0.01)	0.04 (±0.01)	0.07 (±0.03)	0.06 (±0.04)
β-Xylosidase (U ml^{-1})	0.48 (±0.13)	0.07 (±0.03)	0.24 (±0.04)	0.14 (±0.04)
Protease (U ml^{-1})	0.93 (±0.10)	0.73 (±0.16)	1.24 (±0.09)	0.83 (±0.16)
μ_{max} (h^{-1})	0.13 (±0.03)	0.14 (±0.04)	0.14 (±0.04)	0.19 (±0.02)
Q_p^{max}(U ml^{-1} h^{-1})	8.3 (±0.3)	8.4 (±0.1)	18.3 (±0.8)	8.2 (±0.8)
$Y_{p/x}$ (U g^{-1})	30,000 (±4,900)	43,000 (±4,200)	47,000 (±4,900)	25,000 (±700)

FPUs filter paper units, Q_p^{max} maximum volumetric rate of xylanase production, calculated from the maximum slope of the enzyme activity versus time curve, $Y_{p/x}$ specific xylanase yield, units per biomass, μ_{max} maximum specific growth rate

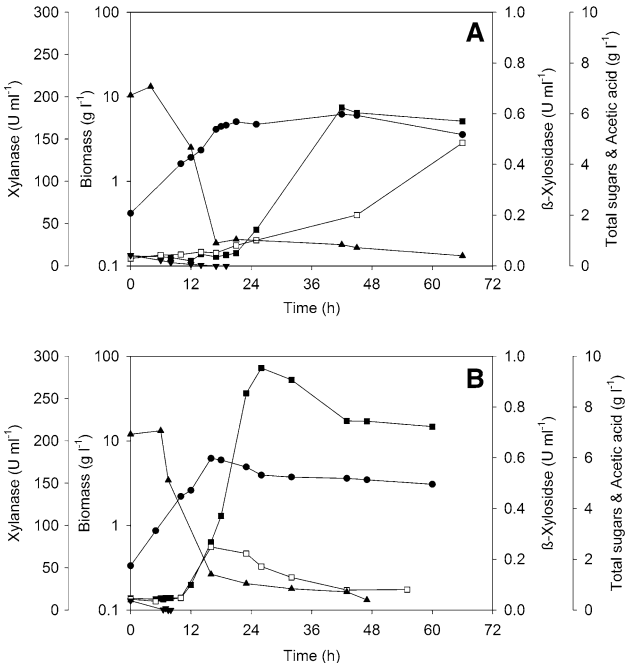

Fig. 3 Typical cultivation profiles of *A. oryzae* NRRL 3485 with xylan as carbon substrate at pH 4.0 (**a**) and pH 7.5 (**b**). Symbols: biomass (*filled circle*), xylanase activity (*filled square*), *β*-xylosidase activity (*open square*), total sugars (*filled triangle*), acetic acid (*filled inverted triangle*)

sample from the culture without pH control also showed the presence of these three xylanase bands.

Effect of agitation rate

Growth parameters of *A. oryzae* at different agitation rates, using the impeller tip velocity as indicator of shear stress, are shown in Table 2. Impeller tip velocities in the range of 1.56–3.12 m s^{-1} were selected for investigation because, in controlling the DOT at ≥25% of saturation during batch cultivation, the stirrer speed operated within this range. Increasing the impeller tip speed from 1.56 to 3.12 m s^{-1} did not have any major effect on the xylanase activity nor on the maximum volumetric rate of xylanase production. When the tip speed exceeded 1.95 m s^{-1}, however, the final biomass concentration decreased slightly, even though the greatest μ_{max} values of 0.23 and 0.265 h^{-1} were recorded at tip speeds of 2.34 and 3.12 m s^{-1}, respectively. The specific xylanase yield was also higher at the higher impeller tip speeds.

Discussion

This study demonstrated that the culture parameters of *A. oryzae* were greatly dependent on the pH. Xylanase production by *A. oryzae* increased with increasing pH to reach a maximum activity at pH 7.5 with both xylan and SSL as respective carbon substrates. Others similarly found that a cultivation pH of up to 7.5 favoured xylanase production by a *Thermomyces lanuginosus* strain [29] and *Aspergillus fischeri* fxn1 grew well over a wide pH range with maximal xylanase activities between pH 6 and 10 [10]. By contrast, *A. fumigatus* AR1 produced the highest xylanase activity at a low pH of 5 [2] and maximal xylanase production by *A. fumigatus* VTT-D-82195 occurred below pH 3.5 [7]. The above observations, therefore, indicate that the optimum pH for xylanase production by filamentous fungi is species or even strain specific.

At pH 4.0 with SSL as carbon substrate, the lag phase was prolonged and lasted up to 51 h until the acetic acid concentration in the culture broth had decreased to minimal levels due to its assimilation by the fungus (Fig. 2a). This indicated that the acetic acid present in the SSL retarded initial growth, despite its relatively low initial concentration of 1.13 g l^{-1} in the culture medium. The inhibitory effect of acetic acid on microbial growth is well documented and is pH-dependent due to the toxicity of the undissociated weak acid [3, 27]. The results obtained with xylan as carbon substrate, however, demonstrated that the relatively high optimal pH for xylanase production in the

Table 2 Growth parameters (mean values of duplicate experiments with standard deviation of the mean) of *A. oryzae* NRRL 3485 in batch culture at a controlled DOT of 25% of saturation and at different stirrer speeds with SSL as carbon feedstock

Parameter	Impeller tip velocity (m/s)			
	1.56	1.95	2.34	3.12
Xylanase (U ml^{-1})	89 (±13)	86 (±7)	94 (±11)	94 (±10)
Biomass (g l^{-1})	3.5 (±0.6)	3.2 (±0.3)	2.6 (±0.3)	2.6 (±0.1)
μ_{max} (h^{-1})	0.21 (±0.01)	0.20 (±0.03)	0.23 (±0.04)	0.27 (±0.05)
Q_P^{max} (U ml^{-1} h^{-1})	2.7 (±0.1)	2.4 (±0.5)	2.6 (±0.01)	2.3 (±0.04)
$Y_{p/x}$ (U g^{-1})	25,000 (±700)	27,000 (±4,200)	36,000 (±0)	36,000 (±5,700)

Q_P^{max} maximum volumetric rate of xylanase production, calculated from the maximum slope of the enzyme activity versus time curve, $Y_{p/x}$ specific xylanase yield, units per biomass, μ_{max} maximum specific growth rate

SSL-based medium was not solely due to the greater inhibition by acetic acid at the lower pH values.

Some reports indicated that xylose was a good inducer of xylanase production [10, 24], whereas others found that xylose repressed xylanase production [14, 32], suggesting that xylose repression of xylanase synthesis was strain specific. However, repression versus induction might also be dependent on the xylose concentration. The cultivation profiles presented in Figs. 2 and 3 show that rapid xylanase production commenced only after the total sugar concentration in the culture broth had decreased to low levels, suggesting derepression of xylanase synthesis at these low sugar concentrations. Because D-xylose constituted over 80% of the total sugars in the SSL, repression of xylanase production was probably a consequence of mainly the D-xylose in the SSL-based medium.

The β-xylosidase activities produced by the *A. oryzae* strain used in this study were comparable to the activities reported for other *Aspergillus* strains such as *A. fumigatus*, *A. terreus* and *A. tamarii* [6, 22]. In filamentous fungi β-xylosidases may be extracellular or cell-bound, whereas in almost all bacteria and yeasts these enzymes are cell-associated [9]. The β-xylosidases of filamentous fungi are also associated with the mycelium during the early phases of growth and may later be released into the medium, either by secretion or as a result of cell lysis, hence the recovery of β-xylosidases in extracellular fractions [25, 36, 30].

The multiplicity of β-1,4-xylanases in filamentous fungi is well known [37]. For example, an *A. niger* culture filtrate was found to contain 15 xylanases, a culture of *Trichoderma viride* 13 and a *Talaromyces emersonii* culture between 11 and 13 xylanases [8, 13]. The zymogram of crude xylanase preparations from the *A. oryzae* strain used in this study revealed three low molecular weight xylanases that were produced across the culture pH range investigated. This is, however, contrary to other reports that *A. fumigatus* AR1 and *T. reesei* RUT C-30 responded to the culture pH by modifying their enzyme production patterns. *A. fumigatus* AR1 produced multiple xylanases at pH 9.0, whereas only a single xylanase was found at pH 5.0 [2]. Similarly, *T. reesei* RUT C-30 produced xylanase I and II at pH 4.0, whereas cultivation at pH 6.0 resulted in the production of xylanase II and III [38]. These findings indicated that some fungi modified their enzyme production patterns according to the culture pH, but this trait also appeared to be strain specific and was not applicable to the strain of *A. oryzae* strain used in this investigaton.

The decrease in xylanase production at high stirrer speeds has been attributed to the effects of hydrodynamic stress, which may cause hyphal disruption and leakage of intracellular compounds [29]. Shear stress as a result of agitation intensity has also been reported to cause morphological as well as physiological changes in some filamentous fungi, resulting in decreased xylanase production [28, 35]. Most studies on the effects of shear stress on enzyme production by fungi used agitation rates as the sole parameter without taking the impeller dimensions into account. This renders meaningful comparisons between studies difficult. The shearing strength may be represented by the impeller tip velocity [18]. The results presented here show that impeller tip velocities within the range of 1.56–3.12 m s^{-1}, equivalent to stirrer speeds of 400–800 rpm, did not have a marked effect on the xylanase activity in the *A. oryzae* culture. These results correlated with the observation that α-amylase and amyloglucosidase production by a recombinant strain of *A. oryzae* were not affected by increasing the agitation rate and that the expression of homologous and heterologous proteins remained independent of stirrer speed in the range of 550–1,000 rpm [1]. There are, however, numerous reports to the contrary. For example, *Thermomyces lanuginosus* SSBP produced the highest xylanase activity with D-xylose as carbon substrate at a low stirrer speed of 400 rpm, whereas higher agitation rates exerted a negative effect on xylanase production [33]. The negative effect of a high agitation rate on xylanase production was also confirmed in respect of *T. lanuginosus* DSM 5826 and *T. lanuginosus* RT9 [29, 21].

Even though the xylanase activity was not affected by agitation intensity in the current study, we observed that at high impeller tip speeds of 2.34 and 3.12 m s^{-1} lower biomass concentrations but slightly higher maximum specific growth rates were obtained than at tip speeds of 1.56 and 1.95 m s^{-1}. These results were consistent with observations on *T. lanuginosus* RT9, which suggested that at low stirrer speeds oxygen limitation slowed down the growth rate but that the concomitant low shear stress aided a higher final biomass concentration, whereas at higher stirrer speeds the oxygen-sufficient conditions facilitated a higher growth rate but that the increased shear stress resulted in lower biomass concentrations [21]. The high specific xylanase yields (U per g biomass) that we observed at high impeller tip speeds of 2.34 and 3.12 m s^{-1} were mainly due to the decreased biomass concentrations, as the volumetric xylanase activities remained fairly constant over the range of impeller tip speeds used in this investigation.

In conclusion, the results presented here clearly demonstrated that xylanase production by *Aspergillus oryzae* NRRL 3485 was greatly affected by the culture pH, with a pH of 7.5 favouring xylanase production in the SSL-based medium, whereas the maximal xylanase activity on xylan was recorded at pH 6 and 7.5. Therefore, the enhanced xylanase production in the SSL-based medium at pH 7.5 was not solely due to the inhibitory action at low pH values of the acetic acid component of the SSL. Cellulase activity was negligible on both these carbon substrates throughout the cultivation pH range. It was also shown that xylanase

activities were independent of impeller tip velocities in the range of 1.56–3.12 m s^{-1}. Furthermore, in agreement with our earlier shake flask experiments [11], the present investigation showed that spent sulphite liquor constituted a suitable cheap carbon feedstock as well as inducer for the production of essentially cellulase-free xylanases by this strain of *A. oryzae*.

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