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# Optimization of process parameters and fermentation strategy for xylanase production in a stirred tank reactor using a mutant *Aspergillus nidulans* strain

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ABSTRACT

The present work studied the optimization of aeration rate, agitation rate and oxygen transfer and the use of various batch fermentation strategies for xylanase production from a recombinant *Aspergillus nidulans* strain in a 3 L stirred tank reactor. Maximum xylanase production of 1250 U/mL with productivity of 313 U/mL/day was obtained under an aeration rate of 2 vvm and an agitation rate of 400 rpm using batch fermentation. The optimum volumetric oxygen transfer coefficient ($k_La$) for efficient xylanase production was found to be 38.6 $h^{-1}$. Fed batch mode and repeated batch fermentation was also performed with $k_La$ was 38.6 $h^{-1}$. Xylanase enzyme productivity increased to 327 with fed batch fermentation and 373 U/mL/day with repeated batch fermentation. Also, maximum xylanase activity increased to 1410 U/mL with fed batch fermentation and 1572 U/mL with repeated batch fermentation.

## 1. Introduction

Xylanases are a class of enzymes that catalyze hydrolysis of xylan, which is a major component of hemicellulose [1]. Xylanases have many crucial applications in industry ranging from food processing to biofuel production [2–7]. Many operation parameters, such as agitation, aeration, temperature and dissolved oxygen concentration must be investigated and optimized to maximize xylanase production from fungi, the major source of xylanases [8]. Agitation and aeration are the most crucial process parameters as they both affect oxygen transfer to cells, which is a decisive factor in the scale up of aerobic fermentation [9]. Oxygen transfer is related to oxygen solubility and diffusion into the broth [10]. Aeration efficiency can be increased by increasing agitation. Proper agitation results in an increase of the gas liquid interface area by disintegrating large air bubbles into many small ones. Agitation also breaks apart mycelial aggregates and thus increases oxygen diffusion into cells [11].

Several previous reports have described production and characterization of an endo-beta-1,4-xylanase from the family GH10 from *Aspergillus fumigatus* var *niveus*, also referred to as AFUMN-GH10 [12–15] by a recombinant *Aspergillus nidulans* strain. Using a recombinant enzyme producing strain often results in easier and more economical purification steps since recombinant strains often only excrete a single protein [16]. Xylanase production by the *A. nidulans* strain was comparable to other xylanase producers [13], and the strain excreted only xylanase [12].

In the *A. nidulans* strain mentioned above, a maltose-induced promoter was used to initiate and promote xylanase production [12]. Maltose is also the carbon source the strain used for protein production; thus, maltose could be subject to substrate inhibition. One cell cultivation method developed to overcome substrate inhibition is fed batch fermentation. Fed batch fermentation involves an initial batch period followed by addition of fresh medium to the reactor until the maximum volume of the reactor is reached. This strategy allows nutrient feeding to be controlled according to metabolic change as expressed as variation in pH, DO % and substrate and by-products concentrations [17–19]. A modification of fed batch strategy, repeated batch fermentation, involves withdrawing part of the old media and replacing it with fresh media to replenish used substrates while keeping the same

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volume [20]. Repeated batch mode increased productivity in a previous xylanase production study compared with batch and fed batch modes [21].

This study aimed to optimize and scale up xylanase production from a recombinant *Aspergillus nidulans* strain with a pyridoxine marker [12] in a stirred tank reactor (STR). The effect of aeration, agitation, and volumetric oxygen mass transfer coefficient ($k_La$) on xylanase production were investigated. Xylanase activities and productivities for the fed batch process and repeated batch process were compared to those from batch fermentation to determine if these strategies could improve the amount of xylanase activity produced and xylanase productivity.

## 2. Materials and methods

### 2.1. Microbial strains, plasmids

*A. nidulans* strain A773 (*pyrG89*; *wA3*; *pyroA4*) was obtained from Fungal Genetic Stock Center (FGSC, Manhattan, KS, USA). This strain is unable to synthesize pyridoxine [22]. The strain was modified as described in [23] to express AFUMN-GH10 [12,13,16,24]. The plasmid used for transformation included a glucoamylase promoter induced by maltose, which allowed overexpression and secretion of AFUMN-GH10 into the media, followed by a tryptophan terminator (*trpCt*) [23].

### 2.2. Inoculum preparation

Spores kept in fungal stock solution (20 % glycerol, 10 % lactose) at −80 °C were thawed and 20 μl were distributed onto a Petri dish containing potato dextrose agar media. Petri dishes were incubated at 37 °C for 2 days. The spores were scraped from the plate and added to 10 mL of distilled water, giving a final concentration of $4 \times 10^8$ spores/mL in the spore inoculum [25]

Cell pellets were prepared by inoculating 0.5 mL spore suspension into 250 mL Erlenmeyer flasks containing 50 mL of preculture media containing glucose, 10; $NaNO_3$, 12; KCl, 2; $MgSO_4$, 0.5; $KH_2PO_4$, 1.5; 1 mL/L 1000× trace element solution (22 g/L $ZnSO_4.7H_2O$, 11 g/L $H_3BO_3$, 5.0 g/L $MnCl_2.7H_2O$, 5.0 g/L $FeSO_4.7H_2O$, 1.6 g/L $CoCl_2.5H_2O$, 1.6 g/L $CuSO_4.5H_2O$, 1.1 g/L $Na_2MoO_4.4H_2O$, 50 g/L $Na_2$-EDTA) and 1 mg/L pyridoxine. The inoculated flasks were incubated in an orbital shaker at 37 °C and 225 rpm for 2 days [13].

### 2.3. Fermentation in a STR

Batch fermentation kinetics were studied in a 3 L STR (Eppendorf BioFlo 115, Hauppage, NY, USA) with a 1.98:1 height: diameter ratio containing 1.5 L of fermentation medium. The fermentation medium has the same composition as the preculture medium plus 120 g/L maltose. Silicone antifoam 204 (Sigma-Aldrich, St. Louis, MO, USA) was added to control foaming. The initial pH was adjusted to 6.5 with 1 M NaOH before autoclaving. A heat sterilizable polarographic oxygen electrode (Mettler Toledo, Columbus, OH, USA) was used to measure dissolved oxygen concentration. Media addition or removal was controlled using a level probe (2 L foam/level sensor kit, Eppendorf). After autoclaving the vessel containing medium at 121 °C, 204.7 kPa for 30 min, the STR was inoculated with 150 mL of pre-culture medium (cell pellets) and operated at 37 °C. To evaluate effect of aeration rate, three runs were conducted using an agitation rate of 400 rpm and an aeration rate 0.5, 1.0 or 2 vvm. To evaluate agitation rate, three runs were conduted using an aeration rate of 2 vvm and an agitation rate of 200, 400 or 600 rpm. Samples were taken daily, centrifuged at 13,000 rpm for 10 min, and used for analysis.

For fed batch fermentation, 500 mL of medium containing 180 g/L maltose and 5 g/L glucose was pulse-fed to 1 L media when enzyme activity started to decrease at both 144 and 240 h. For repeated batch fermentation, 1 L of an initial 1.5 L of fermentation broth was replaced with fresh medium containing 180 g/L maltose and 10 g/L glucose at 144 and 264 h. These times were chosen because enzyme concentration ceased increasing at these times. The agitation speed was 400 rpm and the aeration rate was 2 vvm for both fed batch and repeated batch fermentation.

### 2.4. Volumetric oxygen transfer coefficient ($k_La$) measurement

The unsteady-state method was used to measure $k_La$ in cell free media [20,26]. Nitrogen was sparged into media until dissolved oxygen concentration became zero and then air was sparged until media was saturated with oxygen. Dissolved oxygen concentration variation with time, t, was recorded and $k_La$ was calculated according to the following equation:

$$\ln (C^* - C_L) = \ln (C^* - C^0) - k_La \cdot t \qquad (1)$$

where $C^*$ was saturated dissolved oxygen concentration in liquid phase (mmol/L), $C_L$ was oxygen concentration in liquid phase (mmol/L), $C^0$ was oxygen concentration at t = 0 (mmol/L) (which equaled 0 since all oxygen was purged from the media) and $k_La$ was oxygen transfer coefficient ($h^{-1}$). The $k_La$ was determined by plotting ln ($C^* - C$) against time (t) and determining the slope of the resulting line, which equaled -$k_La$.

### 2.5. Analytical methods and determination of fermentation parameters

Xylanase activity was assayed using beechwood xylan (TCI America, Portland, OR, USA). 0.95 mL of a 1% (w/v) xylan solution in 0.05 M citrate buffer (pH 5) was incubated with 0.05 mL of fermentation medium at 50 °C for 15 min. The reaction was stopped by adding 0.5 mL of DNS reagent to the assay contents. The contents were then boiled in a water bath for 5 min and cooled to room temperature. The absorbance of the assay contents was measured at 575 nm and compared to a substrate control without fermentation medium [27] to determine the amount of reducing sugar in the solution. One international unit (U) of xylanase activity corresponded to the amount of enzyme that catalyzed the release of 1 μmol/min of reducing sugar under the specified assay condition.

Dry cell weight measurements were conducted by filtering a known volume of fermentation medium through a pre-weighed filter (P8 Fisherbrand, Fisher Scientific, Hampton, NH, USA). The filter was then washed with distilled water and dried to constant weight at 60 °C. The remaining cell mass on the filter was determined using an analytical balance. Total protein concentration was assayed using the method described in [28]. Maltose and glucose were determined by HPLC (Dionex Ultimate 3000, Thermo Scientific, Waltham, MA, USA) on an HPX-87 P column (300 mm × 7.8 mm). The eluent was HPLC grade DI-water with a flow rate of 0.6 mL/min at 80 °C. Sugars were measured by a refractive index detector (Shodex RI-101, Tokyo, Japan) and the concentrations were quantified based on a four-level calibration curve of known standards [29]. All assays were performed in triplicate.

## 3. Results and discussion

### 3.1. Effect of different aeration rates on xylanase production

Fig. 1 shows the fermentation kinetics for batch fermentation at 400 rpm and different aeration rates (0.5, 1 and 2 vvm). Increasing

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aeration rate resulted in increased rates of substrate and oxygen consumption and protein and xylanase production. There was more change in fermentation media pH during the growth phase as aeration rates increased. At 48 h pH changed from an initial value of 6.00–5.89, 7.00 and 7.54 with aeration rates of 0.5, 1 and 2 vvm, respectively. This is explained by higher growth and higher metabolism rates at higher aeration rates [30,31]. At the end of fermentation, the recorded pH was 5.70, 6.02 and 6.50 at 0.5, 1 and 2 vvm, respectively. DO% at 24 h was 9, 15 and 26 % at 0.5, 1 and 2 vvm, respectively, and then decreased at 48 h to 3, 1.5 and 0.3 % at 0.5, 1 and 2 vvm, respectively. DO% increased during the stationary and death phases to 4, 7 and 9% at 0.5, 1 and 2 vvm, respectively, at the end of fermentation (Fig. 1A).

Maximum xylanase activities and total protein concentrations were observed at 96 h. Xylanase activities and protein concentrations increased as aeration rate increased. Maximum xylanase activities of 520, 887 and 1250 IU/mL and maximum total protein concentrations of 120, 214 and 300 μg/mL were observed at 0.5, 1 and 2 vvm, respectively (Fig. 1B). The sum of the residual maltose and glucose concentrations at the end of fermentation decreased as aeration rate increased and were 98, 38 and 26 g/L at 0.5, 1 and 2 vvm, respectively (Fig. 1C). An increase in aeration rate generally would enhance the DO level in the growth phase, resulting in an increase cell growth and xylanase production. While cell growth was not measured here, increased xylanase activities and protein concentrations were

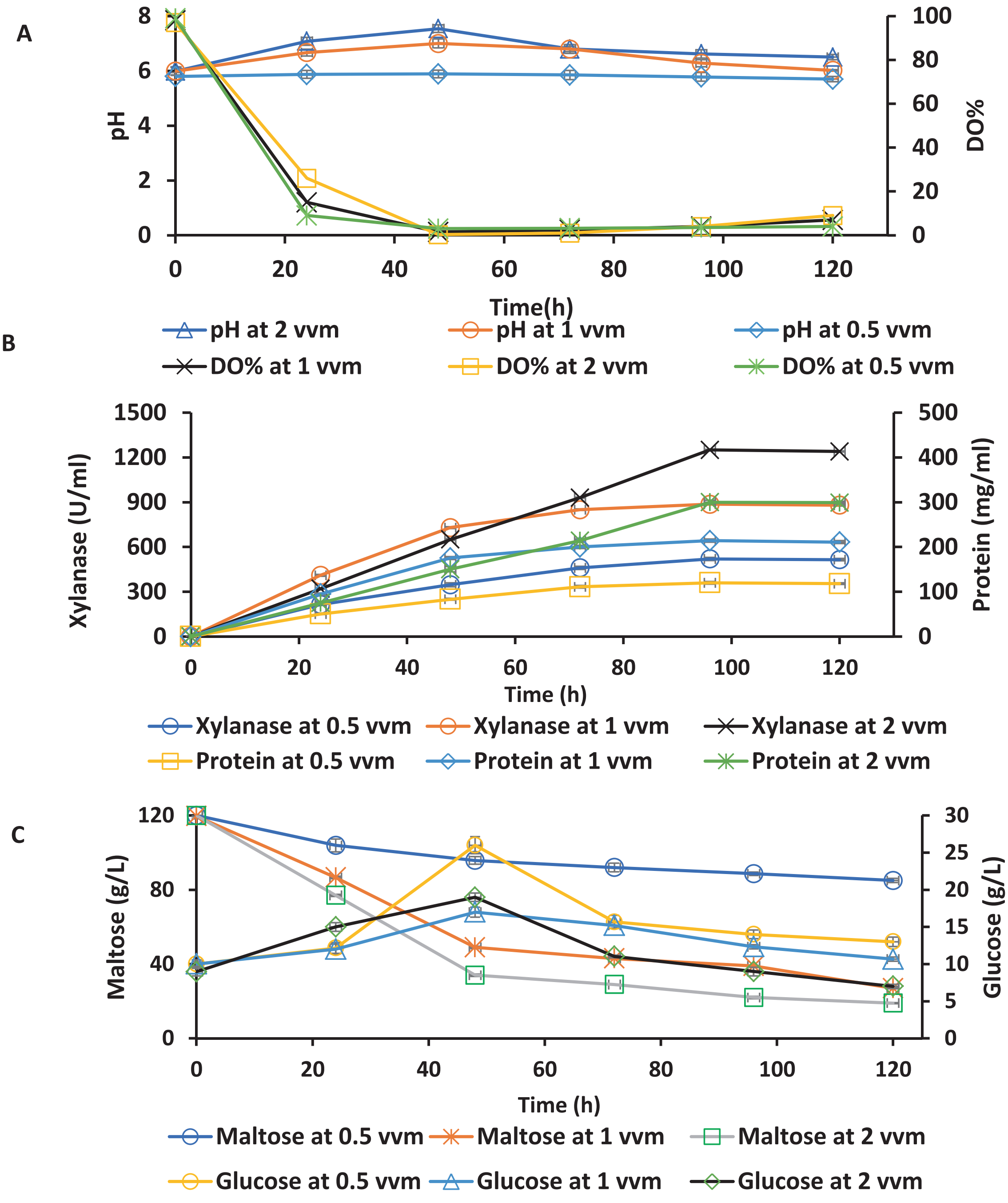

**Fig. 1.** Effects of aeration rate on (A) pH and dissolved oxygen (DO), (B) xylanase activity and protein concentration, and (C) maltose and glucose concentrations during fermentation of *A. nidulans* in a stirred-tank bioreactor inoculated with cell pellets with agitation speed at 400 rpm.

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observed when more oxygen was supplied to the fermenter. DO is one of the most important factors in aerobic fermentation, and any change in DO% can result in considerable changes in cell physiology and metabolism [33]. Previous studies also stated that increasing aeration rate significantly increased xylanase production by *Aspergillus niger* [30,34,35].

### *3.2. Effect of different agitation rates on xylanase production*

Agitation is considered one of the most vital parameters for fermentation conducted in STRs since it controls transfer of oxygen, heat and nutrients from the medium to the microorganism's cells, fragments air into small bubbles to improve gas-liquid contact and prevents mycelia from clumping [9,11,36]. During the first 48 h, the highest pH value of 7.54 was recorded for agitation of 400 rpm followed by 7.15 with 600 rpm and 6.80 for 200 rpm. At the end of fermentation, the recorded pH values were 5.93, 6.50 and 6.17 for 200, 400 and 600 rpm, respectively. DO% at 24 h increased with increasing agitation speed. During the first 24 h, DO% was 18, 26 and 39 % for 200, 400 and 600 rpm, respectively. From 24–48 h, DO% decreased to 4, 0.3 and 2% for 200, 400 and 600 rpm respectively, then from 48 h to the end of the fermentation, DO% increased to 6, 9 and 12 % for 200, 400 and 600 rpm, respectively (Fig. 2A**)**.

Maximum xylanase activities and total protein concentrations were observed at 96 h, which was also observed in the fermentations conducted to study effect of aeration rate. Fig. 2**B** shows that at an agitation speed of 400 rpm, maximum xylanase production was 1250 IU/mL and maximum protein concentration was 300 μg/mL. When agitation rate was increased to 600 rpm, maximum xylanase activity decreased to 995 U/mL and maximum protein concentration decreased to 230 μg/mL. Increase in agitation speeds can cause high shear stress that leads to mycelial rupture destruction of cellular structures which decreases both mycelial growth and enzyme production [37–39]. The lowest enzyme activity of 750 U/mL and the lowest protein concentration of 165 μg/mL were observed at 200 rpm. Lower agitation rates result in reduced mixing in the medium and lower oxygen supply to the microorganism. Ghoshal et al. [32] also observed that decreased agitation rate decreased both fungal growth and enzyme production. Bandaiphet and Prasertsan [40] observed that decreased agitation rate resulted in increased media viscosity and decreased mass transfer. Residual substrate (maltose + glucose) concentrations at the end of fermentation were 39 g/L, 26 g/L and 33 g/L at 200, 400 and 600 rpm, respectively, which supported the observed trend in xylanase activity and protein concentration with lower residual substrate corresponding to higher xylanase activity and protein concentration (Fig. 2C**)**.

### *3.3. Effects of agitation and aeration on $k_La$*

Determination of oxygen transfer inside the STR was carried out by measurement of $k_La$. $k_La$ can be improved by increasing aeration and/or agitation, but only to a certain limit due to the harmful effect of high shear stress [41]. The effect of different agitation speeds and aeration rates on $k_La$ is demonstrated in Fig. 3. The increase of both parameters, in all cases, led to an increase in $k_La$. Fig. 3**A** shows that an aeration rate of 0.5 vvm resulted in $k_La$ values of 5.35, 19.29 and 43.19 $h^{-1}$ at agitation rates of 200, 400 and 600 rpm, respectively. An aeration rate of 1 vvm resulted in $k_La$ values 7.60, 28.93 and 50.78 $h^{-1}$ at agitation rates of 200, 400 and 600 rpm, respectively, and an aeration rate of 2 vvm resulted in $k_La$ values 10.64, 38.55 and 65.19 $h^{-1}$ at agitation rates of 200, 400 and 600 rpm, respectively.

A increase in $k_La$ due to increase of agitation speed was much greater than increase in $k_La$ due to increase of aeration rate; thus, agitation was more effective than aeration for increasing $k_La$ in the reactor used in this study. The recorded $k_La$ at the lowest aeration rate and highest agitation speed, 43.19 $h^{-1}$, was greater than that recorded at the lowest agitation speed and highest aeration rate 10.64 $h^{-1}$. The results are similar to those reported by Fenice et al. [41].

### *3.4. Relationship between $k_La$ and production of xylanase*

In aerobic fermentation oxygen transfer to microbial cells has a significant effect on product formation, which makes $k_La$ an essential parameter to be evaluated in STRs [35]. The highest xylanase activity of 1250 U/mL was attained at $k_La$ of 38.55 $h^{-1}$ where the agitation rate was 400 rpm and the aeration rate was 2 vvm. Increasing kLa from 10.64 $h^{-1}$ at 2 vvm, 200 rpm to 38.55 $h^{-1}$ at 400 rpm lead to an 166 % increase in xylanase activity. At 200 rpm, the stirrer did not load the air flow resulting in low air dispersion and low dissolved oxygen concentration for fungal growth and xylanase production [9,41]. Further, increasing $k_La$ from 38.55 $h^{-1}$ at 400 rpm to 65.19 $h^{-1}$ at 600 rpm reduced xylanase activity from 1250 U/mL to 995 U/mL. This could be explained by the high shear stress in case of high agitation speed, as discussed above.

### *3.5. Fed batch fermentation*

Fed-batch fermentation was conducted by adding fresh medium containing maltose and glucose at 144 and 240 h. Fig. 4**A** shows fermentation profiles for 13 days in a STR inoculated with cell pellets. The pH increased from 5.95 initially to 7.25 at 48 h and then decreased to 6.41 at 120 h. After addition of fresh medium at 144 h, pH was 6.12 then increased to 6.29 at 168 h, after which pH decreased to 5.72 at 216 h. After the second addition of media at 240 h, pH increased again to 6.19 and then to 6.32 at 264 h, after which pH decreased to 5.86 at the end of fermentation. DO was not controlled and decreased from 99 % initially to 0.5 % after 48 h, then increased to 9.3 % at 144 h. After media addition at 144 h, DO decreased to 5.2 % at 168 h, then increased again to 8.0 % at 240 h. After the second media addition at 240 h, DO decreased to 6.1 % at 264 h and then increased to 7.0 % at the end of fermentation. Dos Reis et al. [42] also reported decrease of oxygen concentration after the addition of cellulose during fed batch production of xylanase by *Penicillium echinulatum*. This is due to the recovery of microorganism growth after fresh media addition, which increased oxygen consumption and decreased DO%.

From Fig. 4**B** we can conclude that xylanase and protein production started after 24 h and reached maximum values of 1193 U/mL and 320 μg/mL, respectively, at 96 h. Xylanase productivity was 298 U/mL/d, which was similar to the productivity observed in batch fermentation (313 U/mL/d). After addition of fresh medium at 144 h, xylanase activity and protein concentration decreased to 760 U/mL and 225 μg/mL, respectively, due to dilution. Activity then increased to 1413 U/mL and protein concentration increased to 403 μg/mL at 192 h as fresh nutrients were consumed, resulting in a xylanase productivity of 327 U/mL/d from 96 to 192 h. After the second media addition at 240 h, xylanase activity decreased to 1000 U/mL and protein concentration decreased to 310 μg/mL. Xylanase activity increased to 1300 U/mL and protein concentration increased to 390 μg/mL at 298 h, resulting in a xylanase productivity of 150 U/mL/d from 240 to 298 h.

Maltose concentration decreased from the initial 120.0 g/L to 15.0 g/L at 120 h. After addition of fresh medium at 144 h, maltose increased to 70.0 g/L, and then decreased to 14.1 g/L at 216 h. After the second addition of media at 240 h, maltose concentration

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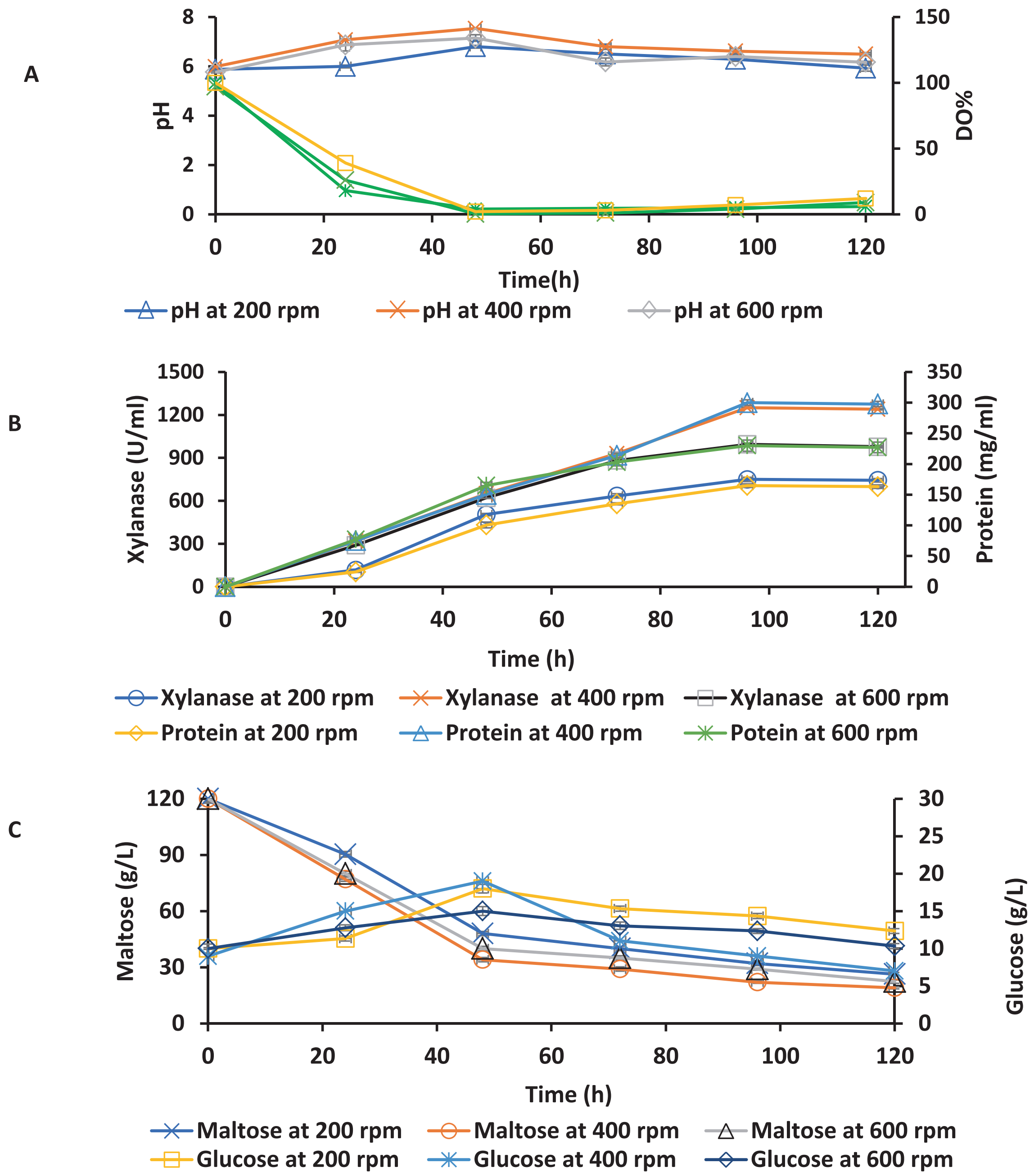

**Fig. 2.** Effects of agitation speed on (A) pH and dissolved oxygen (DO), (B) xylanase activity and protein concentration, and (C) maltose and glucose concentrations during fermentation of *A. nidulans* in a stirred-tank bioreactor inoculated with cell pellets with aeration rate at 2 vvm.

increased to 69.0 g/L, and then decreased to 13.8 g/L at the end of fermentation. Glucose concentration increased from 9.0 g/L at the beginning of fermentation to 18.2 g/L at 48 h due to hydrolysis of maltose by the fungus, then decreased to 8.0 g/L at 120 h [43]. After addition of fresh media at 144 h, glucose increased to 9.6 g/L, increased further to 18.1 g/L at 192 h, then decreased to 13.0 g/L at 216 h. After the second addition of media at 240 h, glucose increased to 14.8 g/L and then increased to 18.8 g/L at 264 h, reaching a value of 12.0 g/L at the end of fermentation (Fig. 4C).

### *3.6. Repeated batch fermentation*

An increase in cell density and enzyme productivity has been shown previously in repeated batch fermentation [21]. This technique is cost effective because productivity and yield can be improved compared to other fermentation modes [44]. To improve enzyme production, fresh media containing maltose and glucose replaced the same volume of old media at set points during batch fermentation (144 and 264 h) that were chosen based on cessation of enzyme production. Fig. 5A shows the fermentation profiles over 14 days in a STR inoculated with cell pellets. The pH increased from 5.87 initially to 7.47 at 48 h and then decreased to 6.26 at 120 h. After the first media replacement at 144 h, pH was 6.00, which increased to 6.17 at 168 h and then decreased to 5.57 at 240 h. After the second media replacement at 264 h, pH was 6.05, increased to 6.15 at 288 h and finally decreased to 5.39 at the end of fermentation. DO was not controlled and decreased rapidly from 100 % initially to 0.6 % at 48 h, then DO increased to 9.0 % at 120 h.

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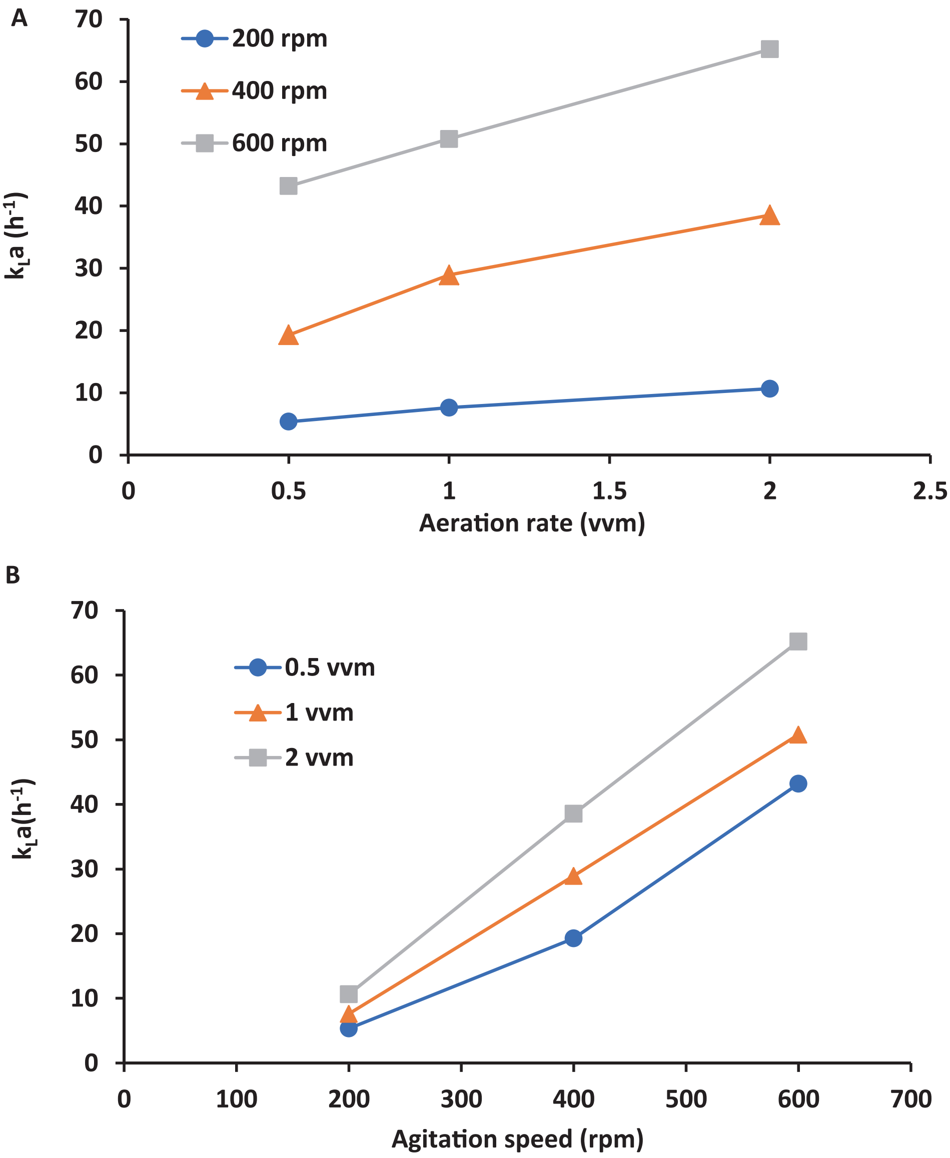

**Fig. 3.** (A) Effect of aeration rate on the volumetric mass transfer coefficient $k_La$ at different agitation speeds and (B) effect of agitation speed on the volumetric mass transfer coefficient $k_La$ at different aeration rates.

After the first media replacement at 144 h, DO decreased to 4.6 % at 168 h, then increased again to 9.1 % at 240 h. After second media replacement at 264 h, DO decreased to 5.9 % at 288 h and then increased to 8.1 % at the end of fermentation.

Maximum values of xylanase activity and protein concentration were 1260 U/mL and 315 μg/mL, respectively, at 96 h for a xylanase productivity of 315 U/mL/day, which was similar to the xylanase productivities observed during batch fermentation and the initial batch phase of fed batch fermentation. After the first media replacement at 144 h, xylanase activity and protein concentration decreased to 453 U/mL and 120 μg/mL, respectively, due to dilution. At 216 h xylanase activity increased to 1571 U/mL and protein concentration increased to 381 μg/mL, which resulted in a xylanase productivity of 373 U/mL/day from 144 h to 216 h. After the second media replacement at 264 h, xylanase activity and protein concentration decreased to 610 U/mL and 139 μg/mL, respectively. At 312 h, xylanase activity increased to 870 U/mL and protein concentration increased to 183 μg/mL, resulting in a xylanase productivity of 130 U/mL/d from 264 h to 312 h.

Maltose concentration decreased from an initial value of 119.3 g/L to 18.3 g/L at 120 h. After the first media replacement at 144 h, maltose concentration was 125.0 g/L, which decreased to 18.0 g/L at 240 h. After the second media replacement at 264 h, maltose concentration was 120.7 g/L, which then decreased to 22.0 g/L at the end of fermentation. Glucose concentration increased from the initial 9.5 g/L to 17.1 g/L at 48 h due to hydrolysis of maltose, and then decreased to 9.0 g/L at 120 h. After the first media replacement at 144 h, glucose was 15.0 g/L and increased further to 22.0 g/L at 192 h due to maltose hydrolysis, and then decreased to 14.0 g/L at 240 h. After the second media replacement at 264 h, glucose concentration was 17.0 g/L, increased to 22.2 g/L at 288 h due to maltose hydrolysis, and then decreased to 16.1 g/L at the end of fermentation (Fig. 5C).

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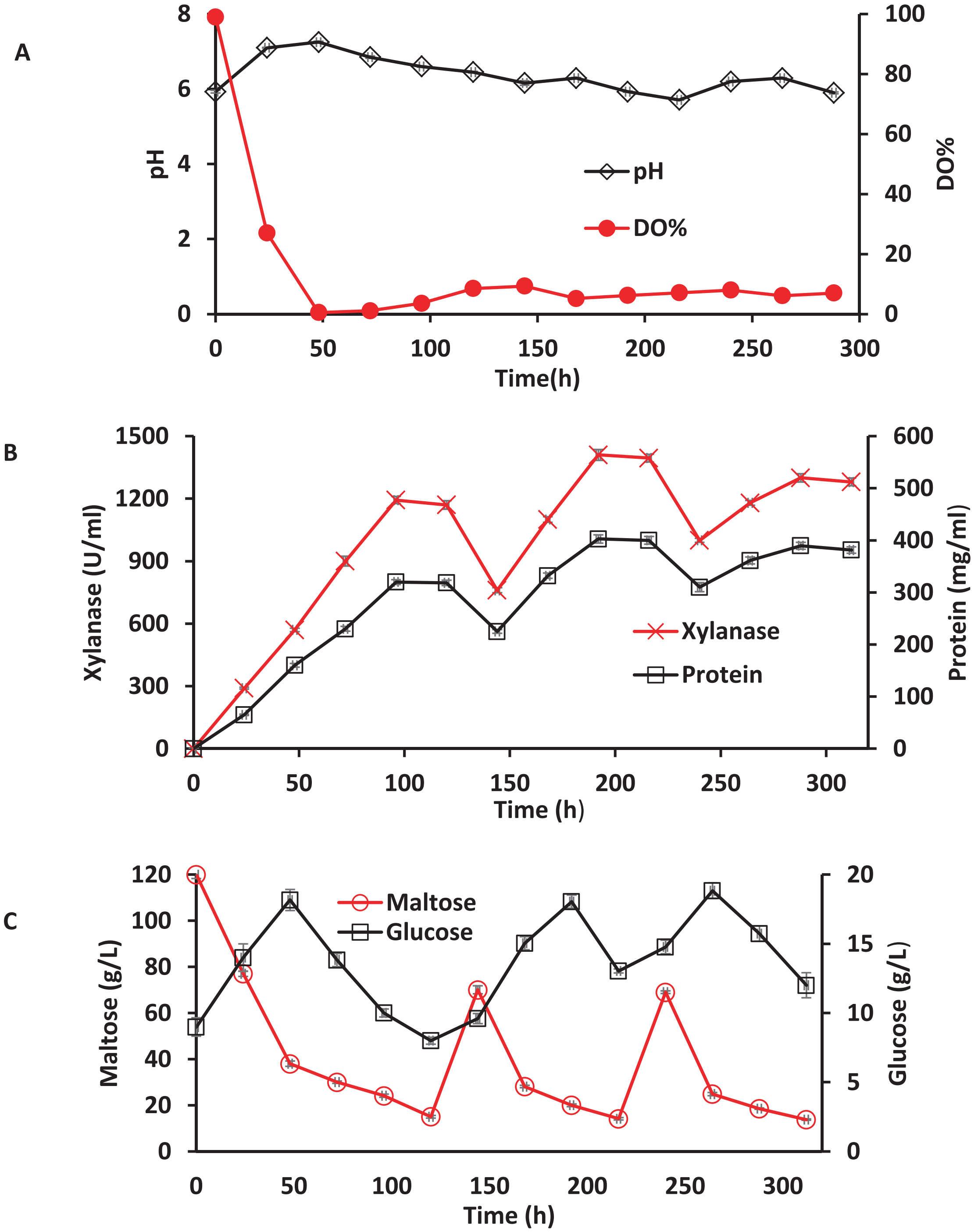

**Fig. 4.** Fed-batch fermentation kinetics of *A. nidulans* in a stirred-tank bioreactor inoculated with cell pellets at 400 rpm and 2 vvm. (A) pH and dissolved oxygen (DO); (B) xylanase activity and protein concentration; (C) maltose and glucose concentrations.

### 3.7. Comparison between different modes of fermentation

The xylanase activity and productivity from different fermentation modes are displayed in Table 1. Xylanase productivities were similar after the first 96 h for all fermentation modes conducted under the same aeration rate (2 vvm) and agitation speed (400 rpm). The mean xylanase activity was 1233 U/mL with a standard deviation of 33 U/mL for the first 96 h of batch fermentation at 2 vvm and 400 rpm, fed batch fermentation and repeated batch fermentation. The mean xylanase productivity for these three fermentations was 309 U/mL/d with a standard deviation of 9 U/mL/d. No additional xylanase activity or protein was produced after 96 h in any of the fermentations. During the second phase of fed batch fermentation, which started when additional media was added at 144 h, a xylanase productivity of 327 U/mL/d was observed, which was a 6% increase compared to

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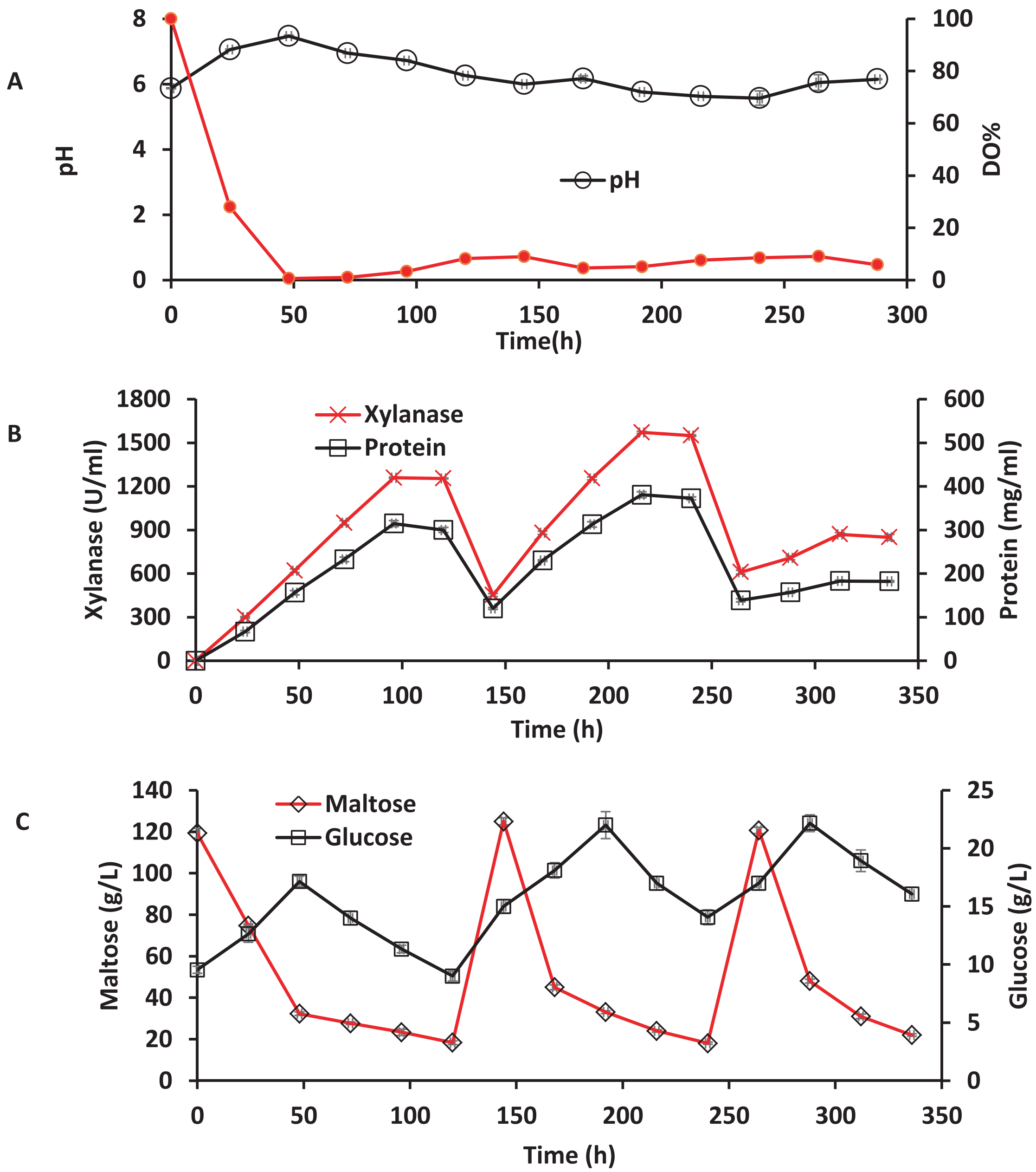

**Fig. 5.** Repeated-batch fermentation kinetics of *A. nidulans* in a stirred-tank bioreactor inoculated with cell pellets at 400 rpm and 2 vvm. (A) pH and dissolved oxygen (DO); (B) xylanase activity and protein concentration; (C) maltose and glucose concentrations.

the initial batch period productivity. During the second phase of fed batch fermentation, which started when media was replaced at 168 h, a xylanase productivity of 373 U/mL/d was observed, which was a 21 % increase compared to the initial batch period productivity and a 14 % increase compared to the second phase of fed batch fermentation. Shang et al. [19] reported that fed batch fermentation increased productivity of xylanase production by *Pichia pastoris*. Dos Reis et al. [42] also reported that the maximum activity of a xylanase from *Penicillium echinulatum* was obtained under fed batch mode. Techapun et al. [45] reported that repeated batch fermentation mode increased productivity of a xylanase from by *Streptomyces* Ab 106. In future work with the *A. nidulans* AFUMN-GH10 strain, the second phase of either fed batch or repeated batch fermentation should be started at 96 h since no additional xylanase activity was produced after the first 96 h. Also, a second media addition or replacement should not be done for either repeated batch or fed batch fermentation as productivity decreased greatly after the second media addition in fed batch and the second media replacement in repeated batch (Table 2).

Many bacteria, yeasts and filamentous fungi can produce xylanases [19,46,47]. Among the filamentous fungi, the genus *Aspergillus* is considered the best for xylanase production [35,48,49]. In this study, a xylanase was produced by recombinant *A. nidulans* in a STR under repeated batch mode showing a high xylanase activity 1571 u/mL and productivity of 373 U/mL/d when

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**Table 1**
Comparison of xylanase production by *A. nidulans* in a STR operated in different modes.

| Fermentation mode | Enzyme activity (U/mL) | Enzyme productivity (U/mL/day) |
|---|---|---|
| **Batch** | 1250 | 313 |
| 0 to 96 h | | |
| **Fed Batch** | 1193 | 298 |
| 1st 96 h | 1410 | 327 |
| 144 to 192 h | 1300 | 150 |
| 240 to 288 h | | |
| **Repeated Batch** | 1260 | 315 |
| 0 to 96 h | 1572 | 373 |
| 168 to 192 h | 870 | 130 |
| 268 to 312 h | | |

**Table 2**
Comparison of xylanase enzyme production in different bioreactors by Aspergillus and other microorganisms under different modes of fermentation.

| Microorganism | Type of reactor | Fermentation mode | Xylanase Activity (U/mL) | Productivity (U/mL/day) | Reference |
|---|---|---|---|---|---|
| *Aspergillus nidulans* | STR | Repeated Batch | 1571 | 373 | This study |
| *Penicillum citrinum* | STR | Batch | 299.51 | 74.87 | [32] |
| *Pichia pastoris* | STR | Fed batch | 560.7 | 140.17 | [19] |
| *Bacillus subtilis* | STR | Batch | 300 | 240 | [47] |
| *Aspergillus niger* | STR | Continuous | 182 | 45.5 | [50] |
| *A. niger* | airlift | Batch | 7 | 1.4 | [35] |
| *A. niger KKS* | Bubble column | Batch | 91 | 18.2 | [51] |
| *Streptomyces* sp. Ab106 | STR | Repeated batch | 32 | 6.4 | [47] |

compared with other studies in literature (Table 2). In addition, using a recombinant enzyme producing strain often results in easier and more economical purification steps since recombinant strains often only excrete a single protein [16].

## 4. Conclusion

This work aimed to study the optimum conditions for xylanase production in a STR using a recombinant *A. nidulans* strain. Oxygen transfer into microbial cells during aerobic bioprocesses strongly affects product formation by influencing metabolic rate. In a STR there are two main factors, aeration and agitation, that influence oxygen transfer rate. It was therefore important to consider the implication of these factors of the volumetric oxygen transfer coefficient ($k_La$). It was shown that high $k_La$ was preferred for enzyme production, but an agitation rate of 600 rpm had a harmful effect on enzyme production due to high shear stress on the production organism, *A. nidulans*. The conditions that resulted in the greatest xylanase activity produced were 400 rpm agitation, 2 vvm aeration rate, and $k_La$ of 38.6 $h^{-1}$. Using fed batch and repeated batch cell cultivation strategies to limit substrate inhibition increased xylanase productivity compared to batch cultivation. Xylanase productivity increased from 309 U/mL/day with batch cultivation to 327 U/mL/day with fed batch and 373 U/mL/day with repeated batch. This work showed that enhanced aeration and agitation combined with a repeated batch cell cultivation mode improved xylanase production from this recombinant *Aspergillus nidulans* strain.

## 5. Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Conflict of interest

The authors do not have any conflict of interest.

## CRediT authorship contribution statement

**Asmaa Abdella:** Conceptualization, Formal analysis, Investigation, Writing - original draft. **Fernando Segato:** Conceptualization, Funding acquisition, Resources, Writing - review & editing. **Mark R. Wilkins:** Conceptualization, Supervision, Funding acquisition, Project administration, Writing - review & editing.

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