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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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## <sup>1</sup> Optimization of process parameters and fermentation strategy for 2 xylanase production in a stirred tank reactor using a mutant Aspergillus <sup>3</sup> nidulans strain

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#### A R T I C L E I N F O

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#### A B S T R A C T

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  $(k<sub>i</sub>a)$  for efficient xylanase production was found to be 38.6 h<sup>-1</sup>. Fed batch mode and repeated batch fermentation was also performed with  $k_{L}$ a was 38.6 h<sup>-1</sup>. 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.

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### 12 1. Introduction

13 Xylanases are a class of enzymes that catalyze hydrolysis of<br>14 years which is a major component of homically local 11 Yulanases <sup>14</sup> xylan, which is a major component of hemicellulose [\[1](#page-9-0)]. Xylanases<br> $\frac{15}{15}$  have many crucial applications in industry ranging from food  $15$  have many crucial applications in industry ranging from food<br> $16$  processing to biofuel production  $[2, 7]$ . Many operation parameters  $^{16}$  processing to biofuel production  $[2-7]$  $[2-7]$ . Many operation param-<br> $^{17}$  eters, such as agitation against temperature and dissolved  $17$  eters, such as agitation, aeration, temperature and dissolved  $18$  express concentration must be investigated and optimized to oxygen concentration must be investigated and optimized to 19 maximize xylanase production from fungi, the major source of  $\frac{20}{20}$  with respect to meet exists process. <sup>20</sup> xylanases [[8\]](#page-10-0). Agitation and aeration are the most crucial process  $\frac{21}{2}$  process are the visit of the magnetic set of the magnetic set of the magnetic set of the set o 21 parameters as they both affect oxygen transfer to cells, which is a<br>22 decisive factor in the scale up of agrebic formentation [0]. Owner 22 decisive factor in the scale up of aerobic fermentation [[9](#page-10-0)]. Oxygen<br>23 december is solited to appear a plubility and different into the harth  $^{23}$  transfer is related to oxygen solubility and diffusion into the broth  $^{24}$ <sup>24</sup> [[10\]](#page-10-0). Aeration efficiency can be increased by increasing agitation.<br><sup>25</sup> **Depart agitation results in an increase of the gas liquid interface** <sup>25</sup> Proper agitation results in an increase of the gas liquid interface  $\frac{26}{1000}$  are hundred integrating large in hubbles integranal and case.  $^{26}$  area by disintegrating large air bubbles into many small ones.<br> $^{27}$  Agitation also broaks apart musclial aggregates and thus increases 27 Agitation also breaks apart mycelial aggregates and thus increases<br> $\frac{28}{2}$  express diffusion into cells [11] oxygen diffusion into cells [[11\]](#page-10-0).

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Several previous reports have described production and  $^{29}$ <br>procterization of an endo-beta-1.4-yylanase from the family  $^{30}$ characterization of an endo-beta-1,4-xylanase from the family  $30$ <br>CH10 from Aspergillus fuminatus var niveus also referred to as GH10 from Aspergillus fumigatus var niveus, also referred to as  $31$ <br>AEUMN CH10 [12, 15] by a recombinant Aspergillus ridulars strain AFUMN-GH10 [\[12](#page-10-0)–15] by a recombinant Aspergillus nidulans strain.  $32$ <br>Using a recombinant enzume producing strain often results in Using a recombinant enzyme producing strain often results in  $33$ <br>axier and more economical purification staps since recombinant <sup>34</sup> easier and more economical purification steps since recombinant <sup>34</sup><br>strains often only excrete a single protein [16]. Yulanase production <sup>35</sup> strains often only excrete a single protein  $[16]$  $[16]$ . Xylanase production  $35$ <br>by the  $A$  pidulars strain was comparable to other wilanase by the A. nidulans strain was comparable to other xylanase  $36$ <br>producers [12] and the strain excreted only vylanase [12] producers [[13\]](#page-10-0), and the strain excreted only xylanase [\[12](#page-10-0)].

In the A. nidulans strain mentioned above, a maltose-induced  $38$ promoter was used to initiate and promote xylanase production  $39$ <br>[12] Maltose is also the sarbon source the strain used for protein  $40$ [\[12](#page-10-0)]. Maltose is also the carbon source the strain used for protein  $\frac{40}{12}$ <br>production: thus maltose could be subject to substante inhibition  $\frac{41}{12}$ production; thus, maltose could be subject to substrate inhibition.  $41$ <br>One cell cultivation method developed to overcome substrate  $42$ One cell cultivation method developed to overcome substrate  $42$ <br>inhibition is fed batch fermentation. Eed batch fermentation  $43$ inhibition is fed batch fermentation. Fed batch fermentation  $^{43}$ <br>involves an initial batch noried followed by addition of fresh  $^{44}$ involves an initial batch period followed by addition of fresh  $\frac{44}{\text{median}}$ medium to the reactor until the maximum volume of the reactor is<br>reached. This strategy allows putrient feeding to be controlled 46 reached. This strategy allows nutrient feeding to be controlled <sup>46</sup><br>according to motabolic change as expressed as variation in pH\_DO 47 according to metabolic change as expressed as variation in pH, DO  $^{47}$ <br> $\%$  and substate and by products concentrations [17, 10]  $\AA$   $^{48}$ % and substrate and by-products concentrations  $[17-19]$  $[17-19]$ . A  $48$ <br>modification of fod batch strategy repeated batch formontation  $49$ modification of fed batch strategy, repeated batch fermentation,  $\frac{49}{100}$ <br>involves withdrawing part of the old media and replacing it with  $\frac{50}{100}$ involves withdrawing part of the old media and replacing it with  $50$ <br>fresh media to replacieb used substrates while keeping the same  $51$ fresh media to replenish used substrates while keeping the same

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 $52$  volume [\[20](#page-10-0)]. Repeated batch mode increased productivity in a<br> $53$  provisus wilapses production study sempared with batch and fed  $53$  previous xylanase production study compared with batch and fed<br> $54$  batch and text<sup>134</sup>  $54$  batch modes [[21](#page-10-0)].

<sup>55</sup> This study aimed to optimize and scale up xylanase production<br> $\frac{56}{2}$  from a recombinant *Acnosilly pidulary* strain with a pyridovine <sup>56</sup> from a recombinant Aspergillus nidulans strain with a pyridoxine<br> $\frac{57}{2}$  expected 121 in a stirred tank reactor (STP). The effect of agration <sup>57</sup> marker [[12](#page-10-0)] in a stirred tank reactor (STR). The effect of aeration,<br> $\frac{58}{2}$  aritation, and volumetric oxygen mass transfer soefficient (k, a) on 58 agitation, and volumetric oxygen mass transfer coefficient ( $k<sub>L</sub>a$ ) on xylanase production were investigated. Xylanase activities and productivities for the fed batch process and repeated batch process  $^{60}$  productivities for the fed batch process and repeated batch process  $^{61}$  were compared to those from batch fermentation to determine if <sup>61</sup> were compared to those from batch fermentation to determine if  $62$  these strategies could improve the amount of vylanse activity  $^{62}$  these strategies could improve the amount of xylanase activity<br> $^{63}$  produced and vylanase productivity produced and xylanase productivity.

### $64$  2. Materials and methods

#### <sup>65</sup> 2.1. Microbial strains, plasmids

<sup>66</sup> A. nidulans strain A773 (pyrG89; wA3; pyroA4) was obtained  $^{67}$  from Fungal Genetic Stock Center (FGSC, Manhattan, KS, USA). This  $^{68}$  strain is unable to synthesize nuridoving [32]. The strain use <sup>68</sup> strain is unable to synthesize pyridoxine [\[22](#page-10-0)]. The strain was<br> $\frac{69}{2}$  and  $\frac{1254}{2}$  are described in [22] to suppose AFUMN GU10  $^{69}$  modified as described in [[23](#page-10-0)] to express AFUMN-GH10  $70$  [[12,13,16](#page-10-0),[24](#page-10-0)]. The plasmid used for transformation included a  $71$  glucoamylase promoter induced by maltose, which allowed  $72$  expressions and secretive of AFUMM GU10 into the madis 72 overexpression and secretion of AFUMN-GH10 into the media,<br> $73 \div 51$ followed by a tryptophan terminator (trpCt)  $[23]$  $[23]$ .

### <sup>74</sup> 2.2. Inoculum preparation

75 Spores kept in fungal stock solution (20 % glycerol, 10 % lactose)<br> $76$  at  $80^{\circ}$ C were thawed and 20 ull were distributed onto a Petri dish <sup>76</sup> at  $-80$  °C were thawed and 20 µ were distributed onto a Petri dish<sup>77</sup> containing potato devtrose agar, media Petri dishes were <sup>77</sup> containing potato dextrose agar media. Petri dishes were<br><sup>78</sup> incubated at 37 °C for 2 days. The spores were scraped from the <sup>78</sup> incubated at 37 °C for 2 days. The spores were scraped from the  $^{79}$  $^{79}$  plate and added to 10 mL<br>80 of distilled water giving

<sup>80</sup> of distilled water, giving a final concentration of  $4 \times 10^8$  spores/<br><sup>81</sup> m<sub>l</sub> in the spore inequlum [25]  $\frac{81}{82}$  mL in the spore inoculum [[25](#page-10-0)]

 $\frac{82}{100}$  Cell pellets were prepared by inoculating 0.5 mL spore<br> $\frac{83}{100}$  suspension into 250 mL Free prepared flasks containing 50 mL of <sup>83</sup> suspension into 250 mL Erlenmeyer flasks containing 50 mL of  $^{84}$  preculture media containing glucose 10: NaNO 12: *NCL* 2: MgSO 84 preculture media containing glucose, 10; NaNO<sub>3</sub>, 12; KCl, 2; MgSO<sub>4</sub>,  $\frac{85}{15}$  0.5; KH<sub>2</sub>PO<sub>4</sub>, 1.5; 1 mL/L 1000 x trace element solution (22 g/L  $\frac{86}{7}$  7nSO, 7H, 0.11 g/L H, RO<sub>2</sub>, 5.0 g/L MnCl, 7H, 0.5.0 g/L  $^{86}$  ZnSO<sub>4</sub>.7H<sub>2</sub>O, 11 g/L H<sub>3</sub>BO<sub>3</sub>, 5.0 g/L MnCl<sub>2</sub>.7H<sub>2</sub>O, 5.0 g/L FeSO<sub>4</sub>.7H<sub>2</sub>O, <br> $^{87}$  1.6 g/L CoCl<sub>2</sub>.5H<sub>2</sub>O, 1.6 g/L CuSO, 5H<sub>2</sub>O, 11 g/L N<sub>32</sub>MoO, 4H<sub>2</sub>O <sup>87</sup> 1.6 g/L CoCl<sub>2</sub>.5H<sub>2</sub>O, 1.6 g/L CuSO<sub>4</sub>.5H<sub>2</sub>O, 1.1 g/L Na<sub>2</sub>MoO<sub>4</sub>.4H<sub>2</sub>O, <sup>88</sup> 50 g/L N<sub>2</sub> ED<sub>3</sub><sup>t</sup></sub> ED<sub>4</sub><sup>t</sup> D<sub>3</sub><sup>L</sup> ED<sub>4</sub><sup>t</sup> D<sub>3</sub><sup>L</sup> ED<sub>4</sub><sup>t</sup> D<sub>3</sub><sup>L</sup> ED<sub>4</sub><sup>t</sup> D<sub>3</sub><sup>L</sup> ED<sub>4</sub><sup>t</sup> D<sub>3</sub><sup>L</sup> ED<sub>4</sub><sup>t</sup> D<sub>3</sub><sup>L</sup> ED<sub>4</sub><sup>t</sup> D<sub></sub> <sup>88</sup> 50 g/L Na<sub>2</sub>-EDTA) and 1 mg/L pyridoxine. The inoculated flasks<br> $^{89}$  uses insubated in an orbital shakes at 27 °C and 225 gap for 2 days <sup>89</sup> were incubated in an orbital shaker at 37 °C and 225 rpm for 2 days<br>90  $\frac{1121}{2}$  $[13]$  $[13]$  $[13]$ .

#### <sup>91</sup> 2.3. Fermentation in a STR

92 Batch fermentation kinetics were studied in a 3 L STR<br>93 (Eppenderf Pielle 115 Hauppage NY USA) with a 109:1 beight: 93 (Eppendorf BioFlo 115, Hauppage, NY, USA) with a 1.98:1 height:<br>94 diameter ratio containing 151 of fermentation medium. The <sup>94</sup> diameter ratio containing 1.5 L of fermentation medium. The fermentation medium has the same composition as the preculture <sup>95</sup> fermentation medium has the same composition as the preculture<br><sup>96</sup> medium plus 120 g/L maltose. Silicone, antifoam 204 (Sigma-<sup>96</sup> medium plus 120 g/L maltose. Silicone antifoam 204 (Sigma-<br><sup>97</sup> Aldrich St Louis MO USA) was added to control foaming The 97 Aldrich, St. Louis, MO, USA) was added to control foaming. The  $^{98}$  initial pH was adjusted to 6.5 with 1 M NaOH before autoclaving A  $^{98}$  initial pH was adjusted to 6.5 with 1 M NaOH before autoclaving. A<br> $^{99}$  host sterilizable pelaregraphic exugen electrode (Mettler Telede  $^{99}$  heat sterilizable polarographic oxygen electrode (Mettler Toledo,  $^{99}$  heat sterilizable polarographic oxygen  $100$  Columbus, OH, USA) was used to measure dissolved oxygen  $101$  concentration Media addition or removal was controlled using a <sup>101</sup> concentration. Media addition or removal was controlled using a<br><sup>102</sup> level probe (21 form/level sensor kit. Eppendorf). After autoclay. <sup>102</sup> level probe (2 L foam/level sensor kit, Eppendorf). After autoclav-<br><sup>103</sup> ing the vessel containing medium at 121 °C 204.7 kPa for 30 min <sup>103</sup> ing the vessel containing medium at  $121 \degree$ C, 204.7 kPa for 30 min,<br><sup>104</sup> the STP was inoculated with 150 mL of pre-culture medium (cell <sup>104</sup> the STR was inoculated with 150 mL of pre-culture medium (cell<br><sup>105</sup> pellets) and operated at 37 °C. To evaluate effect of aeration rate <sup>105</sup> pellets) and operated at 37 °C. To evaluate effect of aeration rate,  $106$  three runs were conducted using an agitation rate of 400 rpm and  $106$  three runs were conducted using an agitation rate of 400 rpm and  $107$  an aeration rate 0.5 1.0 or 2 wm. To evaluate agitation rate three  $107$  an aeration rate 0.5, 1.0 or 2 vvm. To evaluate agitation rate, three<br> $108$  gives user conduted using an aeration rate of 2 using and an <sup>108</sup> runs were conduted using an aeration rate of 2 vvm and an  $\frac{109}{2}$  exitation rate of 200, 400 or 600 runs. Samples were taken daily <sup>109</sup> agitation rate of 200, 400 or 600 rpm. Samples were taken daily,<br> $\frac{110}{2000}$  contributed at 12,000 rpm for 10 min, and used for analysis 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  $112$ <br>where growing artistic started to degree as held 144 and 240 k when enzyme activity started to decrease at both 144 and 240 h.  $113$ <br>Fig. arguested, hitch formations, 11, of an initial 151, of For repeated batch fermentation,  $1 \text{ L}$  of an initial  $1.5 \text{ L}$  of  $114$ <br>formentation broth was replaced with fresh medium containing fermentation broth was replaced with fresh medium containing  $115$ <br>120  $\alpha$ <sup>[[</sup>] maltese and 10  $\alpha$ <sup>[[</sup>] glucese at 144 and 264 h. These times 180 g/L maltose and 10 g/L glucose at 144 and 264 h. These times  $116$ <br>were chosen because on zume concentration coased increasing at were chosen because enzyme concentration ceased increasing at  $117$ <br>these times. The agitation speed was 400 rpm and the agration these times. The agitation speed was 400 rpm and the aeration  $118$ <br>rate was 2 wm for both fed batch and repeated batch rate was 2 vvm for both fed batch and repeated batch  $119$ <br>fermentation 120 fermentation.

### 2.4. Volumetric oxygen transfer coefficient  $(k<sub>L</sub>a)$  measurement  $121$

The unsteady-state method was used to measure  $k<sub>L</sub>a$  in cell free  $122$ <br>Intervalse Different was sparted into media until dissolved  $123$ media  $[20,26]$  $[20,26]$  $[20,26]$  $[20,26]$  $[20,26]$ . Nitrogen was sparged into media until dissolved  $[223]$ <br>oxygen concentration became zero and then air was sparged until  $[24]$ oxygen concentration became zero and then air was sparged until  $124$ <br>modia was saturated with owner. Dissolved owner concentration  $125$ media was saturated with oxygen. Dissolved oxygen concentration  $125$ <br>societies with time to was presented and he was relaxited  $126$ variation with time, t, was recorded and  $k<sub>L</sub>$ a was calculated  $126$ <br>according to the following countion according to the following equation:

$$
\ln (C^* - C_L) = \ln (C^* - C^0) - k_L a \cdot t \tag{1}
$$

where  $C^*$  was saturated dissolved oxygen concentration in liquid  $129$ <br>abase (mmal(I) C, was oxygen concentration in liquid phase  $131$ phase (mmol/L),  $C_L$  was oxygen concentration in liquid phase  $131$ <br>(mmol/L)  $C^0$  was oxygen concentration at  $t = 0$  (mmol/L) (which  $132$ (mmol/L),  $C^0$  was oxygen concentration at  $t = 0$  (mmol/L) (which  $132$ <br>could 0 cince all oxygen was pureed from the modia) and k a was equaled 0 since all oxygen was purged from the media) and  $k<sub>L</sub>$ a was  $134$  oxygen transfer coefficient (h<sup>-1</sup>). The  $k<sub>L</sub>$ a was determined by  $134$ plotting ln  $(C^* - C)$  against time (t) and determining the slope of  $135$ <br>the resulting line which equaled k a the resulting line, which equaled  $-k<sub>L</sub>a$ .

#### <sup>137</sup> 2.5. Analytical methods and determination of fermentation parameters

Xylanase activity was assayed using beechwood xylan (TCI  $139$ <br>parisa Bortland OB USA) 0.05 mJ of a 1% (wh) what solution in America, Portland, OR, USA). 0.95 mL of a  $1\%$  (w/v) xylan solution in  $140$ <br>0.05 M, citrate, buffer (pH, 5), was incubated with 0.05 mL of 0.05 M citrate buffer (pH 5) was incubated with 0.05 mL of  $^{141}$ <br>formantation modium at  $50\%$  for 15 min. The reaction was stanned  $^{142}$ fermentation medium at 50 °C for 15 min. The reaction was stopped  $142$ <br>by adding 0.5 mJ of DNS reagent to the assay sontonts. The sentents by adding 0.5 mL of DNS reagent to the assay contents. The contents  $143$ <br>were then holled in a water hath for 5 min and cooled to ream were then boiled in a water bath for 5 min and cooled to room  $144$ <br>tomporature. The absorbance of the assay contents was moasured at temperature. The absorbance of the assay contents was measured at  $145$ <br>575 nm and compared to a substante control without formontation 575 nm and compared to a substrate control without fermentation  $146$ <br>medium [27] to determine the amount of reducing sugar in the medium [[27](#page-10-0)] to determine the amount of reducing sugar in the  $147$ <br>solution. One international unit (II) of vylanase activity corres solution. One international unit (U) of xylanase activity corre-<br>sponded to the amount of enzyme that catalyzed the release of sponded to the amount of enzyme that catalyzed the release of  $149$ <br>1.1 mol/min of reducing sugar under the specified assay condition 1  $\mu$ mol/min of reducing sugar under the specified assay condition.  $\frac{150}{\mu}$ <br>Dry cell weight measurements were conducted by filtering a

Dry cell weight measurements were conducted by filtering a state of the parameters of the parame known volume of fermentation medium through a pre-weighed  $^{152}$ <br>filter (DS Fisherbrand, Eisher Scientific, Hampton, NH, USA), The  $^{153}$ filter (P8 Fisherbrand, Fisher Scientific, Hampton, NH, USA). The  $153$ <br>filter was then washed with distilled water and dried to constant  $154$ filter was then washed with distilled water and dried to constant  $154$ <br>weight at  $60\degree C$ . The remaining cell mass on the filter was  $155$ weight at  $60^{\circ}$ C. The remaining cell mass on the filter was  $155$ <br>determined using an applytical balance Total protein concentra  $156$ determined using an analytical balance. Total protein concentra-<br>tion was assayed using the method described in  $[281 \text{ Maltose and}]$   $[157 \text{ Maltose}]$ tion was assayed using the method described in [\[28\]](#page-10-0). Maltose and  $157$ <br>glucose were determined by HPLC (Dioney Ultimate 2000, Thermo  $158$ glucose were determined by HPLC (Dionex Ultimate 3000, Thermo  $158$ <br>Scientific Maltham MA USA) on an HPX 87 B column 159 Scientific, Waltham, MA, USA) on an HPX-87P column  $159$ <br>(200 mm  $\cdot$  7.8 mm) The elucativize UPLC grade PL unter with a  $160$ (300 mm  $\times$  7.8 mm). The eluent was HPLC grade DI-water with a 160 flow rate of 0.6 mL/min at 80 °C. Sugars were measured by a 161 refractive index detector (Shodey PL 101 Televe Japan) and the 162 refractive index detector (Shodex RI-101, Tokyo, Japan) and the  $162$ <br>concentrations were quantified based on a four lavel calibration  $163$ concentrations were quantified based on a four-level calibration  $163$ <br>gurus of known standards [20], All assays were performed in  $164$ curve of known standards [\[29](#page-10-0)]. All assays were performed in  $164$ <br>triplicate. triplicate. The contraction of t

### **3. Results and discussion 166**

#### <sup>167</sup> 3.1. Effect of different aeration rates on xylanase production

Fig. 1 shows the fermentation kinetics for batch fermentation at  $168$ <br>2 nm and different seration rates (0.5, 1 and 2 nm) kereasing  $169$ <sup>169</sup> 400 rpm and different aeration rates (0.5, 1 and 2 vvm). Increasing

#### A. Abdella et al. / Biotechnology Reports xxx (2019) e00457 33

<span id="page-3-0"></span>170 aeration rate resulted in increased rates of substrate and oxygen<br>171 aeration and oxygential and released resolution. There were  $^{171}$  consumption and protein and xylanase production. There was  $172$  more change in fermentation media pH during the growth phase as  $173$ 173 aeration rates increased. At 48 h pH changed from an initial value<br>174 of 6.00, 5.80, 7.00 and 7.54 with aeration rates of 0.5, 1 and 2 wm 174 of 6.00–5.89, 7.00 and 7.54 with aeration rates of 0.5, 1 and 2 vvm,<br>175 corportively. This is explained by higher growth and higher <sup>175</sup> respectively. This is explained by higher growth and higher  $\frac{176}{20.211}$  at the end of 176 metabolism rates at higher aeration rates [\[30,31](#page-10-0)]. At the end of the recorded  $nH$  was 5.70, 6.02 and 6.50 at 0.5, 1 and 177 fermentation, the recorded pH was 5.70, 6.02 and 6.50 at 0.5, 1 and 1<br>178 2 wm respectively DO% at 24 b was 9.15 and 26 % at 0.5, 1 and 2 <sup>178</sup> 2 vvm, respectively. DO% at 24 h was 9, 15 and 26 % at 0.5, 1 and 2<br><sup>179</sup> www. respectively, and then decreased at 48 h to 3, 1.5 and 0.3 % at <sup>179</sup> vvm, respectively, and then decreased at 48 h to 3, 1.5 and 0.3 % at  $^{179}$  of 5.1 and 2 wm, respectively, DO% increased during the stationary <sup>180</sup> 0.5, 1 and 2 vvm, respectively. DO% increased during the stationary<br><sup>181</sup> and death phases to 4.7 and 9% at 0.5.1 and 2 wm, respectively. at <sup>181</sup> and death phases to 4, 7 and 9% at 0.5, 1 and 2 vvm, respectively, at  $182$  the end of fermentation (Fig. 14) the end of fermentation (Fig. 1A).

Maximum xylanase activities and total protein concentra-<br> $\frac{183}{184}$ tions were observed at 96 h. Xylanase activities and protein  $184$ <br>separate in proceed as a continuous in proceed Marinum  $185$ concentrations increased as aeration rate increased. Maximum  $185$ <br>unlessed as thirties of 520, 997 and 1250 What and maximum  $186$ xylanase activities of 520, 887 and 1250 IU/mL and maximum  $186$ <br>total protoin concentrations of 120, 214 and 200 ug/mL wore  $187$ total protein concentrations of 120, 214 and 300  $\mu$ g/mL were<br>observed at 0.5, 1 and 2 year respectively (Fig. 1P). The sum of 188 observed at 0.5, 1 and 2 vvm, respectively (Fig. 1B). The sum of  $188$ <br>the recidual maltese and glucese concentrations at the end of  $189$ the residual maltose and glucose concentrations at the end of  $189$ <br>fermentation decreased as aeration rate increased and were  $98$   $190$ fermentation decreased as aeration rate increased and were 98,  $190$ <br>38, and 26 g(L at 0.5, 1, and 2, wm, respectively (Fig. 1C), Ap. 191 38 and 26 g/L at 0.5, 1 and 2 vvm, respectively (Fig. 1C). An  $^{191}$ <br>increase in cention atts spaceally used appear the DO layel in  $^{192}$ increase in aeration rate generally would enhance the DO level in  $192$ <br>the growth phase resulting in an increase cell growth and  $193$ the growth phase, resulting in an increase cell growth and  $193$ <br>unlarges anglusting While sell grouth was not measured began  $x$ ylanase production. While cell growth was not measured here,  $194$ <br>increased wilance activities and protein concentrations were  $195$ 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.

#### 4 A. Abdella et al. / Biotechnology Reports xxx (2019) e00457

<sup>196</sup> observed when more oxygen was supplied to the fermenter. DO is  $\frac{197}{2}$  one of the most important fectors in agreebig fermentation, and any <sup>197</sup> one of the most important factors in aerobic fermentation, and any  $198$  shown in  $DQ^{\alpha}$  are would in approximately shown in all <sup>198</sup> change in DO% can result in considerable changes in cell <sup>199</sup> physiology and metabolism [\[33](#page-10-0)]. Previous studies also stated that  $\frac{200}{100}$  increasing against the significantly increased vylanges produc-<sup>200</sup> increasing aeration rate significantly increased xylanase produc-<br> $^{201}$  tion by *Aeparaillys niger* [20.24.25] tion by Aspergillus niger [\[30](#page-10-0),[34,35](#page-10-0)].

### <sup>202</sup> 3.2. Effect of different agitation rates on xylanase production

203 Agitation is considered one of the most vital parameters for<br>204 fermentation conducted in STRs since it controls transfer of  $^{204}$  fermentation conducted in STRs since it controls transfer of  $^{205}$  express heat and putrients from the medium to the micro- $205$  oxygen, heat and nutrients from the medium to the micro-<br> $206$  organism's cells fragments air into small bubbles to improve gas- $206$  organism's cells, fragments air into small bubbles to improve gas-<br> $207$  liquid contact and prevents mycelia from clumping [9.11.36] <sup>207</sup> liquid contact and prevents mycelia from clumping [\[9,11](#page-10-0),[36\]](#page-10-0).<br><sup>208</sup> During the first 48 h the bighest pH value of 7.54 was recorded for <sup>208</sup> During the first 48 h, the highest pH value of 7.54 was recorded for  $\frac{209}{200}$  aritation of 400 rpm followed by 7.15 with 600 rpm and 6.80 for  $^{209}$  agitation of 400 rpm followed by 7.15 with 600 rpm and 6.80 for  $^{210}$  and  $^{200}$  rpm At the and of formantation, the recepted pH values were <sup>210</sup> 200 rpm. At the end of fermentation, the recorded pH values were<br><sup>211</sup> 5.02.6.50 and 6.17 for 200, 400 and 600 rpm, respectively, DO% at <sup>211</sup> 5.93, 6.50 and 6.17 for 200, 400 and 600 rpm, respectively. DO% at  $\frac{212}{24}$  at high respected with increasing activities apped. During the first <sup>212</sup> 24 h increased with increasing agitation speed. During the first  $\frac{213}{24}$  24 h DO<sup>o</sup> was 18, 26 and 20  $\%$  for 200, 400 and 600 rpm 213 24 h, DO% was 18, 26 and 39 % for 200, 400 and 600 rpm,<br>214 reconstitutive Fram 24, 48 h, DO% decreased to 4.0.2 and 2% for 200 <sup>214</sup> respectively. From 24–48 h, DO% decreased to 4, 0.3 and 2% for 200,<br><sup>215</sup> 400 and 600 gpm respectively, then from 48 h to the and of the <sup>215</sup> 400 and 600 rpm respectively, then from 48 h to the end of the<br><sup>216</sup> formantation DO<sup>V</sup> increased to 6, 0 and 12  $\%$  for 200, 400 and <sup>216</sup> fermentation, DO% increased to 6, 9 and 12 % for 200, 400 and  $^{217}$  600 gas association (Fig. 24) <sup>217</sup> 600 rpm, respectively [\(Fig.](#page-5-0) 2A).

<sup>218</sup> Maximum xylanase activities and total protein concentrations<br><sup>219</sup> Were observed at 06 b, which was also observed in the <sup>219</sup> were observed at 96 h, which was also observed in the  $\frac{220}{28}$  formontations conducted to study offect of agration rate  $\frac{125}{28}$ <sup>220</sup> fermentations conducted to study effect of aeration rate. [Fig.](#page-5-0) 2**B**<br><sup>221</sup> chours that at an agitation speed of 400 rpm maximum vulanage <sup>221</sup> shows that at an agitation speed of 400 rpm, maximum xylanase<br><sup>222</sup> production was 1250  $\frac{U}{m}$  and maximum protein concentration <sup>222</sup> production was 1250 IU/mL and maximum protein concentration<br><sup>223</sup> was 300 ug/mL When agitation rate was increased to 600 rpm <sup>223</sup> was 300  $\mu$ g/mL. When agitation rate was increased to 600 rpm,<br><sup>224</sup> maximum vulanase activity decreased to 995 U/mL and maximum <sup>224</sup> maximum xylanase activity decreased to 995 U/mL and maximum<br><sup>225</sup> motein, concentration, decreased, to 230 ug/mL. Increase, in <sup>225</sup> protein concentration decreased to  $230 \mu$ g/mL. Increase in  $\frac{226}{\mu}$  agitation speeds can cause bigh shear stress that leads to mycelial  $\frac{226}{227}$  agitation speeds can cause high shear stress that leads to mycelial  $\frac{227}{227}$  runture destruction of collular structures which despeces both <sup>227</sup> rupture destruction of cellular structures which decreases both  $^{228}$  musclial groupt and on production  $^{127}$   $^{201}$ . The lowest <sup>228</sup> mycelial growth and enzyme production  $[37-39]$  $[37-39]$ . The lowest<br><sup>229</sup> enzyme activity of 750 U/mJ and the lowest protein concentration <sup>229</sup> enzyme activity of 750 U/mL and the lowest protein concentration<br><sup>230</sup> of 165 ug/mL were observed at 200 rpm. Lower agitation rates <sup>230</sup> of 165  $\mu$ g/mL were observed at 200 rpm. Lower agitation rates<br><sup>231</sup> result in reduced mixing in the medium and lower oxygen supply <sup>231</sup> result in reduced mixing in the medium and lower oxygen supply<br><sup>232</sup> to the micrograpism Choshal et al. <sup>[22]</sup> also observed that  $^{232}$  to the microorganism. Ghoshal et al. [\[32](#page-10-0)] also observed that  $^{233}$  decreased agitation rate decreased both fungal growth and  $233$  decreased agitation rate decreased both fungal growth and  $234$  enzyme production Bandainhet and Prasertsan [40] observed <sup>234</sup> enzyme production. Bandaiphet and Prasertsan  $[40]$  $[40]$  observed<br> $^{235}$  that degreesed exitation rate resulted in increased media viscosity. <sup>235</sup> that decreased agitation rate resulted in increased media viscosity<br><sup>236</sup> and decreased mass transfer. Besidual substate (maltese) glu <sup>236</sup> and decreased mass transfer. Residual substrate (maltose + glu-<br><sup>237</sup> associates the same of formatities were 20  $\sigma$ <sup>[[</sup>] 26  $\sigma$ <sup>[[</sup>] <sup>237</sup> cose) concentrations at the end of fermentation were 39 g/L, 26 g/L<br> $^{238}$  and 22 g/L, the 200, 400 and 600 gaps are a stingly subjek supported <sup>238</sup> and 33 g/L at 200, 400 and 600 rpm, respectively, which supported<br> $^{239}$  the sharmed trand in urlange estimity and protein consentration <sup>239</sup> the observed trend in xylanase activity and protein concentration<br><sup>240</sup> with lower residual substrate corresponding to higher vulanase <sup>240</sup> with lower residual substrate corresponding to higher xylanase<br><sup>241</sup> activity and protein concentration (Fig. 2C) activity and protein concentration [\(Fig.](#page-5-0)  $2C$ ).

### <sup>242</sup> 3.3. Effects of agitation and aeration on  $k_1a$

243 Determination of oxygen transfer inside the STR was carried out  $244$  by mosquement of  $k \geq k$  a can be improved by increasing aeration <sup>244</sup> by measurement of  $k<sub>L</sub>a$ .  $k<sub>L</sub>a$  can be improved by increasing aeration  $\frac{245}{\text{mol/s}}$  and/or agitation but only to a certain limit due to the barmful <sup>245</sup> and/or agitation, but only to a certain limit due to the harmful<br><sup>246</sup> effect of high shear stress [41]. The effect of different agitation <sup>246</sup> effect of high shear stress [[41](#page-10-0)]. The effect of different agitation  $\frac{247}{\text{speeds}}$  and aeration rates on k.a is demonstrated in Fig. 3. The <sup>247</sup> speeds and aeration rates on  $k<sub>L</sub>a$  is demonstrated in [Fig.](#page-6-0) 3. The  $^{248}$  increase of both parameters in all cases led to an increase in  $k<sub>L</sub>a$ <sup>248</sup> increase of both parameters, in all cases, led to an increase in  $k<sub>L</sub>a$ .<br><sup>249</sup> Eig <sup>24</sup> bows that an aeration rate of 0.5 wm resulted in k-avalues <sup>249</sup> [Fig.](#page-6-0) 3A shows that an aeration rate of 0.5 vvm resulted in k<sub>L</sub>a values<br>250 of 5.35, 19.29, and 43.19.b<sup>-1</sup>, at agitation rates of 200, 400, and <sup>250</sup> of 5.35, 19.29 and 43.19 h<sup>-1</sup> at agitation rates of 200, 400 and  $^{251}$  600 rpm respectively. An aeration rate of 1 wm resulted in k a <sup>251</sup> 600 rpm, respectively. An aeration rate of 1 vvm resulted in  $k_L a$ <br><sup>252</sup> values 7.60, 28.93 and 50.78 h<sup>-1</sup> at agitation rates of 200, 400 and<br><sup>253</sup> 600 rpm, respectively, and an aeration rate of 2 vym resulted in <sup>253</sup> 600 rpm, respectively, and an aeration rate of 2 vvm resulted in k<sub>L</sub>a values 10.64, 38.55 and 65.19 h<sup>-1</sup> at agitation rates of 200, 400 and <br><sup>255</sup> 600 npm respectively.  $^{255}$  600 rpm, respectively.

<sup>256</sup> A increase in  $k<sub>L</sub>a$  due to increase of agitation speed was much  $^{257}$  are atom increase in  $k$  a due to increase of againstmental thus greater than increase in  $k<sub>L</sub>$ a due to increase of aeration rate; thus, agitation was more effective than aeration for increasing  $k<sub>L</sub>$  a in the 258 reactor used in this study. The recorded  $k<sub>L</sub>a$  at the lowest aeration  $^{259}$ rate and highest agitation speed, 43.19 h<sup>-1</sup>, was greater than that  $10^{260}$ Fecorded at the lowest agitation speed and highest aeration rate  $261$ <br>10.64 k<sup>-1</sup> The reculte are similar to those reported by Fenice at  $21$  $10.64$  h<sup>-1</sup>. The results are similar to those reported by Fenice et al.  $262$ <sup>263</sup> [[41](#page-10-0)].

### 3.4. Relationship between  $k_1a$  and production of xylanase  $264$

In aerobic fermentation oxygen transfer to microbial cells has a  $^{265}$ <br> $m:$  figure of set as a modust formation, which makes has an  $^{266}$ significant effect on product formation, which makes  $k<sub>L</sub>a$  an essential parameter to be evaluated in STRs [\[35\]](#page-10-0). The highest  $267$  wild parameter to be evaluated in STRs [35]. The highest  $268$ xylanase activity of 1250 U/mL was attained at  $k<sub>L</sub>a$  of 38.55 h<sup>-1</sup> <sup>268</sup> where the agitation rate was 400 rpm and the aeration rate was  $2^{269}$ <br>www.lncreasing kLa from 10.64 h<sup>-1</sup> at 2 www.200 rpm to 38.55 h<sup>-1</sup> 270 vvm. Increasing kLa from 10.64 h<sup>-1</sup> at 2 vvm, 200 rpm to 38.55 h<sup>-1</sup>  $^{270}$ <br>at 400 rpm lead to an 166  $\%$  increase in vylanase activity. At 271 at 400 rpm lead to an 166 % increase in xylanase activity. At  $^{271}$ <br>200 rpm the stirrer did not load the air flow resulting in low air  $^{272}$ 200 rpm, the stirrer did not load the air flow resulting in low air  $\frac{272}{273}$ dispersion and low dissolved oxygen concentration for fungal  $273$ <br>growth and wilapses production [0.41]. Eurther, increasing  $k_2 = 274$ growth and xylanase production [\[9,41](#page-10-0)]. Further, increasing  $k<sub>L</sub>$  a<br>from 38.55 h<sup>-1</sup> at 400 rpm to 65.19 h<sup>-1</sup> at 600 rpm reduced <sup>275</sup><br>whenese activity from 1250 U/mL to 005 U/mL This gould be xylanase activity from 1250 U/mL to 995 U/mL. This could be  $^{276}$ <br>cyrelained by the bigh shear stress in axes of bigh agitation spaced  $^{277}$ explained by the high shear stress in case of high agitation speed,  $\frac{277}{278}$ as discussed above.

### <sup>279</sup> 3.5. Fed batch fermentation

Fed-batch fermentation was conducted by adding fresh 280<br>odium containing malters and glucose at 144 and 240 h 281 medium containing maltose and glucose at 144 and 240 h.  $^{281}$ <br>Fig. 40 shows fermentation profiles for 13 days in a STR  $^{282}$ [Fig.](#page-7-0) 4A shows fermentation profiles for 13 days in a STR  $^{282}$ <br>inoculated with cell pellets. The pH increased from 5.95 initially  $^{283}$ inoculated with cell pellets. The pH increased from 5.95 initially  $120h$  to  $7.25$  at  $48h$  and then decreased to 6.41 at 120 h, After addition  $284$ to 7.25 at 48 h and then decreased to 6.41 at 120 h. After addition  $^{284}$ <br>of fresh medium at 144 h all type 6.13 then increased to 6.29 at  $^{285}$ of fresh medium at 144 h, pH was 6.12 then increased to 6.29 at  $^{285}$ <br>169 h, fter which all degreesed to 5.73 at 216 h, fter the second  $^{286}$ 168 h, after which pH decreased to 5.72 at 216 h. After the second  $^{286}$ <br>addition of media at 240 h, pH increased again to 6.19 and then to  $^{287}$ addition of media at 240 h, pH increased again to 6.19 and then to  $\frac{287}{6.32}$  at 264 h, after which nH decreased to 5.86 at the end of  $\frac{288}{6.32}$ 6.32 at 264 h, after which pH decreased to 5.86 at the end of  $^{288}$ <br>fermentation. DO was not controlled and decreased from 00  $\frac{\alpha}{4}$   $^{289}$ fermentation. DO was not controlled and decreased from 99  $\%$  289<br>initially to 0.5  $\%$  after 48 h, then increased to 9.3  $\%$  at 144 h, After 290 initially to 0.5 % after 48 h, then increased to 9.3 % at 144 h. After  $\frac{290}{2}$ <br>media addition at 144 h. DO decreased to 5.2 % at 168 h, then  $\frac{291}{2}$ media addition at 144 h, DO decreased to 5.2 % at 168 h, then  $^{291}$ <br>increased again to 8.0 % at 240 h. After the second media addition  $^{292}$ increased again to 8.0 % at 240 h. After the second media addition  $\frac{292}{25}$  at 240 h. DO decreased to 6.1 % at 264 h and then increased to 7.0  $\frac{293}{25}$ at 240 h, DO decreased to 6.1 % at 264 h and then increased to 7.0  $^{293}$ <br>% at the end of fermentation. Dec Beie at al. [42] also reported  $^{294}$ % at the end of fermentation. Dos Reis et al.  $[42]$  $[42]$  $[42]$  also reported  $294$ <br>degrees of ourgen concentration after the addition of callulate  $295$ decrease of oxygen concentration after the addition of cellulose  $^{295}$ <br>during fod batch production of vulnages by Popicillium schinu during fed batch production of xylanase by *Penicillium echinu-*<br>*latum* This is due to the resovery of misrographic growth after  $297$ *latum*. This is due to the recovery of microorganism growth after  $297$ <br>fresh modia addition which increased overan consumption and  $298$ <sup>298</sup> fresh media addition, which increased oxygen consumption and decreased DO%. 299<br>From Fig. 4**P** We can conclude that wilances and protein 300

From [Fig.](#page-7-0) 4**B** we can conclude that xylanase and protein  $300$ <br>oduction started after 34 h and reached maximum values of 1103  $301$ production started after 24 h and reached maximum values of 1193 and  $\frac{301}{302}$ U/mL and 320  $\mu$ g/mL, respectively, at 96 h. Xylanase  $302$ <br>productivity was 208 U/mL/d which was similar to the  $303$ productivity was 298 U/mL/d, which was similar to the  $^{303}$ <br>productivity observed in batch fermontation (212 U/mL/d) After  $^{304}$ productivity observed in batch fermentation (313 U/mL/d). After  $304$ <br>addition of fresh medium at 144 h, vylanase activity and protein  $305$ addition of fresh medium at 144 h, xylanase activity and protein  $305$ <br>concentration decreased to 760 U/mL and 225 ug/mL respectively  $306$ concentration decreased to 760 U/mL and 225  $\mu$ g/mL, respectively,  $306$ <br>due to dilution, activity then increased to 1412 U/mL and protein due to dilution. Activity then increased to 1413 U/mL and protein  $307$ <br>concentration increased to 403 u.g/mL at 192 b as fresh putrients  $308$ concentration increased to 403  $\mu$ g/mL at 192 h as fresh nutrients  $308$ <br>were consumed, resulting in a vulanase productivity of 327 U/mL were consumed, resulting in a xylanase productivity of 327 U/mL/  $309$ <br>d, from 96 to 192 b, After the second media addition at 240 b d from 96 to 192 h. After the second media addition at 240 h,  $310$ <br>sylance activity decreased to 1000 U/mL and protein concentra  $311$ xylanase activity decreased to 1000 U/mL and protein concentra-<br>tion decreased to 310 u.g/mL. Yulanase activity increased to 1300 and 312 tion decreased to 310  $\mu$ g/mL. Xylanase activity increased to 1300  $^{312}$ <br> $H/m$  and protein concentration increased to 300  $\mu$ g/mL at 208 b U/mL and protein concentration increased to 390  $\mu$ g/mL at 298 h,<br>resulting in a vylanase productivity of 150 U/mL/d from 240 to  $314$ resulting in a xylanase productivity of 150 U/mL/d from 240 to  $314$ <br>215 <sup>315</sup> 298 h.

Maltose concentration decreased from the initial  $120.0 g/L$  to  $316$ <br> $9 g/L$  at 120 b. After addition of fresh modium at 144 b. maltose  $317$ 15.0 g/L at 120 h. After addition of fresh medium at 144 h, maltose  $317$ <br>increased to 70.0 g/L, and then decreased to 14.1 g/L at 216 h, After  $318$ increased to 70.0 g/L, and then decreased to 14.1 g/L at 216 h. After  $\frac{318}{12}$ <br>the second addition of media at 240 h, maltose concentration  $\frac{319}{12}$ the second addition of media at 240 h, maltose concentration

A. Abdella et al. / Biotechnology Reports xxx (2019) e00457 55

<span id="page-5-0"></span>

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.

<sup>320</sup> increased to 69.0 g/L, and then decreased to 13.8 g/L at the end of<br><sup>321</sup> fermontation Clusses concentration increased from 0.0 g/L at the <sup>321</sup> fermentation. Glucose concentration increased from  $9.0 g/L$  at the<br><sup>322</sup> beginning of formortation to 18.2 g/L at 48 b due to hydrolygic of <sup>322</sup> beginning of fermentation to 18.2 g/L at 48 h due to hydrolysis of<br><sup>323</sup> maltose by the fungus, then decreased to 8.0 g/L at 120 b [42]. After  $\frac{323}{324}$  maltose by the fungus, then decreased to 8.0 g/L at 120 h [\[43\]](#page-10-0). After  $\frac{324}{3}$  addition of fresh media at 144 h glucose increased to 9.6 g/L <sup>324</sup> addition of fresh media at 144 h, glucose increased to  $9.6 g/L$ ,<br>325 increased further to 181 g/L at 102 h, then decreased to 12.0 g/L at <sup>325</sup> increased further to 18.1 g/L at 192 h, then decreased to 13.0 g/L at  $\frac{326}{216}$  at  $\frac{326}{2$ <sup>326</sup> 216 h. After the second addition of media at 240 h, glucose<br><sup>327</sup> increased to 14.8 g/L and then increased to 18.8 g/L at 26.4 h  $\frac{327}{28}$  increased to  $\frac{14.8 \text{ g}}{L}$  and then increased to  $\frac{18.8 \text{ g}}{L}$  at 264 h, reaching a value of 12.0  $g/L$  at the end of fermentation [\(Fig.](#page-7-0) 4C).

### <sup>329</sup> 3.6. Repeated batch fermentation

 $330$  An increase in cell density and enzyme productivity has been<br> $331$  shown previously in repeated batch fermentation [21] This shown previously in repeated batch fermentation  $[21]$  $[21]$  $[21]$ . This technique is cost effective because productivity and yield can be  $332$ <br>improved compared to other formantation modes [44]. To improve improved compared to other fermentation modes  $[44]$  $[44]$ . To improve  $333$ <br>on zume production, fresh modia containing maltese and glucose enzyme production, fresh media containing maltose and glucose  $334$ <br>replaced the same volume of old media at set points during batch replaced the same volume of old media at set points during batch  $335$ <br>formontation (144 and 264 b) that were chosen based on cossation fermentation (144 and 264 h) that were chosen based on cessation  $337$ <br>of anzume production. Fig. 5A shows the fermentation profiles of enzyme production. [Fig.](#page-8-0) 5A shows the fermentation profiles  $337$ <br>over 14 days in a STB inoquiated with call pallate. The pH increased over 14 days in a STR inoculated with cell pellets. The pH increased  $338$ <br>from 5.87 initially to 7.47 at 48 b, and then decreased to 6.36 at from 5.87 initially to 7.47 at 48 h and then decreased to 6.26 at  $339$ <br>120 h After the first media replacement at 144 h pH was 6.00 120 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  $341$ <br>240 h After the second media replacement at 264 h pH was 6.05 240 h. After the second media replacement at 264 h, pH was 6.05,  $342$ <br>increased to 6.15 at 288 h and finally degreesed to 5.20 at the end of increased to 6.15 at 288 h and finally decreased to 5.39 at the end of  $343$ <br>formantation. DO was not controlled and decreased maidly from fermentation. DO was not controlled and decreased rapidly from  $344$ <br>100 % initially to 0.6 % at 48 h than DO increased to 0.0 % at 120 h  $100\%$  initially to 0.6 % at 48 h, then DO increased to 9.0 % at 120 h.

<span id="page-6-0"></span>6 A. Abdella et al. / Biotechnology Reports xxx (2019) e00457



Fig. 3. (A) Effect of aeration rate on the volumetric mass transfer coefficient  $k<sub>L</sub>$  at different agitation speeds and (B) effect of agitation speed on the volumetric mass transfer coefficient kLa at different aeration rates.

346 After the first media replacement at 144 h, DO decreased to 4.6 % at 347 at 8 k then increased again to 0.1 % at 240 h, After second modia <sup>347</sup> 168 h, then increased again to 9.1 % at 240 h. After second media<br><sup>348</sup> replacement at 264 b. DO decreased to 5.0 % at 288 h and then <sup>348</sup> replacement at 264 h, DO decreased to 5.9 % at 288 h and then<br><sup>349</sup> increased to 8.1 % at the end of formantation  $\frac{349}{350}$  increased to 8.1 % at the end of fermentation.

 $\frac{350}{251}$  Maximum values of xylanase activity and protein concentra-<br> $\frac{351}{251}$  tion were 1260 U/mL and 215 u.g/mL respectively at 06 b for a  $\frac{351}{252}$  tion were 1260 U/mL and 315  $\mu$ g/mL, respectively, at 96 h for a  $\frac{352}{353}$  xylanase productivity of 315 U/mL/day, which was similar to the  $\frac{353}{353}$  yylanase productivities observed during batch fermentation and  $353$  xylanase productivities observed during batch fermentation and  $354$  the initial batch phase of fed batch fermentation. After the first  $354$  the initial batch phase of fed batch fermentation. After the first<br> $355$  media replacement at 144 h vylanase activity and protein  $355$  media replacement at 144 h, xylanase activity and protein<br> $356$  concentration decreased to 453 U/mI and 120 ug/mI respec-<sup>356</sup> concentration decreased to 453 U/mL and 120  $\mu$ g/mL, respec-<br><sup>357</sup> tively due to dilution At 216 h xylanase activity increased to 1571 <sup>357</sup> tively, due to dilution. At 216 h xylanase activity increased to 1571<br><sup>358</sup> UlmL and protein concentration increased to 381 ug/mL which <sup>358</sup> U/mL and protein concentration increased to 381  $\mu$ g/mL, which<br><sup>359</sup> resulted in a valanase productivity of <sup>272 U/mL</sup>/day from 144 h to <sup>359</sup> resulted in a xylanase productivity of 373 U/mL/day from 144 h to<br>360 **116 h** After the second media replacement at 264 h wilanase  $\frac{360}{216}$  216 h. After the second media replacement at 264 h, xylanase<br> $\frac{361}{2}$  activity and protein concentration decreased to 610 U/mL and  $\frac{361}{120}$  activity and protein concentration decreased to 610 U/mL and  $\frac{362}{120}$  and recrease its value of the system of the syst  $139 \mu g/mL$ , respectively. At 312 h, xylanase activity increased to 870 U/mL and protein concentration increased to  $183 \mu\text{g/mL}$ ,  $363 \text{ resulting in a}$ resulting in a xylanase productivity of 130 U/mL/d from 264 h to  $364$ <br>365 <sup>365</sup> 312 h.

Maltose concentration decreased from an initial value of  $366$ <br> $32 \frac{\pi}{4}$  to  $18.2 \frac{\pi}{4}$  at 120 b. After the first modia replacement 119.3 g/L to 18.3 g/L at 120 h. After the first media replacement  $367$ <br>at 144 h malters concentration was 125.0 g/L which decreased to at 144 h, maltose concentration was  $125.0$  g/L, which decreased to  $368$ <br>18.0 g/L, at 240 b, After the second modia replacement at 264 b 18.0 g/L at 240 h. After the second media replacement at 264 h,  $369$ <br>maltese concentration was 120.7 g/L, which then decreased to maltose concentration was 120.7 g/L, which then decreased to  $370$ <br>22.0 g/L, at the end of fermentation. Glucose concentration 22.0 g/L at the end of fermentation. Glucose concentration  $371$ <br>increased from the initial 9.5 g/L to 171 g/L at 48 h due to increased from the initial  $9.5 g/L$  to  $17.1 g/L$  at  $48 h$  due to  $372$ <br>hydrolysis of maltose, and then decreased to  $9.0 g/L$  at  $120 h$  hydrolysis of maltose, and then decreased to  $9.0 \text{ g/L}$  at 120 h.  $373$ <br>After the first media replacement at 144 h glucose was 15.0 g/L and After the first media replacement at 144 h, glucose was 15.0 g/L and  $374$ <br>increased further to 22.0 g/L at 192 h, due to maltose hydrolysis increased further to 22.0 g/L at 192 h due to maltose hydrolysis,  $\frac{375}{376}$ <br>and then decreased to 14.0 g/L at 240 h, After the second media and then decreased to  $14.0 g/L$  at 240 h. After the second media  $376$ <br>replacement at 264 h. glucose concentration was  $170 g/L$  in replacement at 264 h, glucose concentration was  $17.0 \text{ g/L}$ , in-<br>crossed to 22.2 g/L, it 288 h due to maltose bydrolygic and then creased to 22.2 g/L at 288 h due to maltose hydrolysis, and then  $\frac{378}{379}$ decreased to 16.1 g/L at the end of fermentation [\(Fig.](#page-8-0) 5C).

A. Abdella et al. / Biotechnology Reports xxx (2019) e00457 7

<span id="page-7-0"></span>

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.

#### <sup>380</sup> 3.7. Comparison between different modes of fermentation

 $381$  The xylanase activity and productivity from different fermen-<br> $382$  tation modes are displayed in Table 1. Xylanase productivities were  $\frac{382}{383}$  tation modes are displayed in [Table](#page-9-0) 1. Xylanase productivities were<br> $\frac{383}{383}$  similar after the first 96 h for all fermentation modes conducted  $\frac{383}{384}$  similar after the first 96 h for all fermentation modes conducted<br> $\frac{384}{384}$  under the same aeration rate (2 wm) and agitation speed  $\frac{384}{100}$  under the same aeration rate (2 vvm) and agitation speed<br> $\frac{385}{100}$  (400 rpm). The mean xylanase activity was 1233 U/mL with a  $\frac{385}{36}$  (400 rpm). The mean xylanase activity was 1233 U/mL with a  $\frac{386}{36}$  standard deviation of 33 U/mL for the first 96 b of batch standard deviation of 33 U/mL for the first 96 $h$  of batch fermentation at 2 vvm and 400 rpm, fed batch fermentation and 387<br>repeated batch fermentation. The mean vylanase productivity for 388 repeated batch fermentation. The mean xylanase productivity for  $388$ <br>these three fermentations was 309 U/mL/d with a standard  $389$ these three fermentations was 309 U/mL/d with a standard  $389$ <br>deviation of 9 U/mL/d. No additional vylanase activity or protein  $390$ deviation of 9 U/mL/d. No additional xylanase activity or protein  $\frac{390}{391}$ <br>was produced after 96 h in any of the fermentations. During the  $\frac{391}{391}$ was produced after 96 h in any of the fermentations. During the  $\frac{391}{392}$ second phase of fed batch fermentation, which started when  $392$ <br>additional media was added at 144 h a xylanase productivity of  $393$ additional media was added at 144 h, a xylanase productivity of  $393$ <br>227 Um I d was observed which was a  $5\%$  increase compared to  $394$  $327$  U/mL/d was observed, which was a 6% increase compared to

<span id="page-8-0"></span>8 A. Abdella et al. / Biotechnology Reports xxx (2019) e00457



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.

 $395$  the initial batch period productivity. During the second phase of  $396$  fod, batch formontation, which started when modia was  $\frac{396}{397}$  fed batch fermentation, which started when media was<br> $\frac{397}{397}$  replaced at 168 h a wilapace productivity of 272 U/mL/d was <sup>397</sup> replaced at 168 h, a xylanase productivity of 373 U/mL/d was<br><sup>398</sup> observed which was a 21 % increase compared to the initial <sup>398</sup> observed, which was a 21 % increase compared to the initial<br><sup>399</sup> batch period productivity and a 14 % increase compared to the <sup>399</sup> batch period productivity and a 14 % increase compared to the  $\frac{400}{2}$  second, phase of fed batch fermentation. Shang et al. [19]  $\frac{400}{401}$  second phase of fed batch fermentation. Shang et al. [[19](#page-10-0)]  $\frac{401}{401}$  reported that fed batch fermentation increased productivity of  $^{401}$  reported that fed batch fermentation increased productivity of  $^{402}$  vylanase production by *Pichia nastoris*. Dos Reis et al. [42] also  $^{402}$  xylanase production by Pichia pastoris. Dos Reis et al. [[42](#page-10-0)] also<br> $^{403}$  reported that the maximum activity of a vulanase from Penicillium  $^{403}$  reported that the maximum activity of a xylanase from *Penicillium*<br> $^{404}$  echinulatum was obtained under fed batch mode. Techanup et al.  $\frac{404}{405}$  echinulatum was obtained under fed batch mode. Techapun et al.  $\frac{405}{406}$  [[45](#page-10-0)] reported that repeated batch fermentation mode increased<br> $\frac{406}{406}$  productivity of a vylance from by Streptomyces Ab 106. In future 406 productivity of a xylanase from by *Streptomyces* Ab 106. In future  $407$  such with the 4 midulane AEUMN GU10 strain, the second phase work with the A. nidulans AFUMN-GH10 strain, the second phase

of either fed batch or repeated batch fermentation should be  $408$ <br>started at 06 b since no additional vulnages activity was produced started at 96 h since no additional xylanase activity was produced  $409$ <br>ofter the first 06 h, Also, a second modia addition or replacement after the first 96 h. Also, a second media addition or replacement  $410$ <br>should not be done for either repeated batch or fed batch should not be done for either repeated batch or fed batch  $411$ <br>fermentation as productivity decreased greatly after the second fermentation as productivity decreased greatly after the second  $412$ <br>media addition in fed batch and the second media replacement in media addition in fed batch and the second media replacement in  $413$ <br>and the second media replacement in repeated batch ([Table](#page-9-0) 2). 414<br>Many bacteria yearsts and filamentous fungi can produce 415

Many bacteria, yeasts and filamentous fungi can produce  $415$ <br>|anases  $119.46.471$  Among the filamentous fungi the xylanases  $[19,46,47]$  $[19,46,47]$  $[19,46,47]$  $[19,46,47]$ . Among the filamentous fungi, the  $416$ <br>converted the same density of the best for wilances production genus Aspergillus is considered the best for xylanase production  $417$ <br> $125,48,401$  In this study a vulanase was produced by resemblant [[35,48,49](#page-10-0)]. In this study, a xylanase was produced by recombinant  $^{418}$ <br>A nidulary in a STP under repeated batch mode changes a high  $^{419}$ A. nidulans in a STR under repeated batch mode showing a high  $419$ <br>whenever activity 1571 v/ml and preductivity of 272 U/ml (d when xylanase activity 1571 u/mL and productivity of 373 U/mL/d when

### A. Abdella et al. / Biotechnology Reports xxx (2019) e00457 9

#### <span id="page-9-0"></span>Table 1

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



Table 2

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



421 compared with other studies in literature (Table 2). In addition,  $422$  critics a recombinent engines are distinguished after results in  $\frac{422}{423}$  using a recombinant enzyme producing strain often results in  $\frac{423}{423}$  excient and more economical purification stans since recombinant  $\frac{423}{424}$  easier and more economical purification steps since recombinant  $\frac{424}{424}$ strains often only excrete a single protein  $[16]$  $[16]$ .

### <sup>425</sup> 4. Conclusion

426 This work aimed to study the optimum conditions for xylanase<br>427 and usting in a STP using a recombinant A pidular setting Outcom  $^{427}$  production in a STR using a recombinant A. nidulans strain. Oxygen<br> $^{428}$ <sup>428</sup> transfer into microbial cells during aerobic bioprocesses strongly<br><sup>429</sup> effects graduat formation by influencing matchelia gate. In a STD  $^{429}$  affects product formation by influencing metabolic rate. In a STR<br> $^{430}$  $\frac{430}{431}$  there are two main factors, aeration and agitation, that influence  $^{431}$  oxygen transfer rate. It was therefore important to consider the  $^{432}$  implication of these fectors of the volumetric curves transfer  $^{432}$  implication of these factors of the volumetric oxygen transfer <sup>433</sup> coefficient (k<sub>L</sub>a). It was shown that high k<sub>L</sub>a was preferred for  $\frac{434}{2}$  continuous production but an agitation rate of 600 rpm had a harmful  $^{434}$  enzyme production, but an agitation rate of 600 rpm had a harmful<br> $^{435}$  effect an enzyme production due to high charge stress on the <sup>435</sup> effect on enzyme production due to high shear stress on the  $\frac{436}{436}$  exclusion organism A nidulary. The conditions that resulted in  $^{436}$  production organism, A. nidulans. The conditions that resulted in  $^{437}$  the greatest vulnase activity produced were 400 rpm agitation 2 <sup>437</sup> the greatest xylanase activity produced were 400 rpm agitation, 2<br><sup>438</sup> www.agration.rate.and ka of 38.6 h<sup>-1</sup> Using fed batch and repeated  $^{438}$  vvm aeration rate, and k $_{\rm L}$ a of 38.6 h $^{-1}$ . Using fed batch and repeated 439 batch cell cultivation strategies to limit substrate inhibition<br>440 increased with an enductivity compared to batch sultivation  $^{440}$  increased xylanase productivity compared to batch cultivation.<br> $^{441}$  Yylanase productivity increased from  $^{200}$  U/mJ/day with batch 441 Xylanase productivity increased from 309 U/mL/day with batch<br>442 cultivation to 327 U/mL/day with fed batch and 373 U/mL/day with  $^{442}$  cultivation to 327 U/mL/day with fed batch and 373 U/mL/day with  $^{443}$  repeated batch. This work showed that enhanced aeration and  $^{443}$  repeated batch. This work showed that enhanced aeration and  $^{444}$  aritation combined with a repeated batch cell cultivation mode  $\frac{444}{445}$  agitation combined with a repeated batch cell cultivation mode<br> $\frac{445}{445}$  improved vylanase production from this recombinant Aspergillus  $\frac{445}{446}$  improved xylanase production from this recombinant Aspergillus  $\frac{446}{446}$ nidulans strain.

#### 447 5. Declaration of interests

448 The authors declare that they have no known competing<br>449 Geographic theoretic arguments and the first could have  $\frac{449}{450}$  financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### **Conflict of interest** 451

The authors do not have any conflict of interest.  $452$ 

### <sup>453</sup> CRediT authorship contribution statement

**Asmaa Abdella:** Conceptualization, Formal analysis, Investiga-<br>B. Writing original draft **Fernando Segato:** Conceptualization 455 tion, Writing - original draft. **Fernando Segato:** Conceptualization,  $455$ <br>Eunding acquisition, Pesources Writing, review & editing **Mark P** 456 Funding acquisition, Resources, Writing - review & editing. **Mark R.** 456<br>Wilking: Concentualization, Supervision, Funding, acquisition, 457 **Wilkins:** Conceptualization, Supervision, Funding acquisition, 457 Project administration Writing a review & editing 458 Project administration, Writing - review & editing.

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### References **Additional Additional A**

- [1] E. Topakas, P. Katapodis, D. Kekos, B.J. Macris, P. Christakopoulos, Production  $\frac{1}{2}$  and partial characterization of xylanase by Sporotrichum thermophile under  $\frac{468}{2}$  and partial characterization of xylanase by Sporotrichum thermophile under  $\frac{468}{2}$ solid-state fermentation, World J. Microbiol. Biotechnol. 19 (2003) 195–198.<br>[2] K.S. Kumar, A. Manimaran, K. Permaul, S. Singh, Production of β-xylanase by a
- [2] K.S. Kumar, A. Manimaran, K. Permaul, S. Singh, Production of <sup>β</sup>-xylanase by <sup>a</sup> <sup>470</sup> Thermomyces lanuginosus MC <sup>134</sup> mutant on corn cobs and its application in biobleaching of bagasse pulp, J. Biosci. Bioeng. 107 (2009) 494–498, doi:[http://](http://dx.doi.org/10.1016/j.jbiosc.2008.12.020) 472<br>[dx.doi.org/10.1016/j.jbiosc.2008.12.020](http://dx.doi.org/10.1016/j.jbiosc.2008.12.020).<br>[3] M.L.T.M. Polizeli, A.C.S. Rizzatti, R. Monti, H.F. Terenzi, J.A. Jorge, D.S. Amorim,
- M.L.T.M. Polizeli, A.C.S. Rizzatti, R. Monti, H.F. Terenzi, J.A. Jorge, D.S. Amorini, 473<br>Xylanases from fungi: properties and industrial applications, Appl. Microbiol. 474<br>Ristasheel C7 (2005) 577 501 Biotechnol. 67 (2005) 577-591.
- [4] M. Azin, R. Moravej, D. Zareh, Production of xylanase by Trichoderma  $\frac{1}{2}$  longibrachiatum on a mixture of wheat bran and wheat straw: optimization of  $\frac{475}{2}$  or the straight of wheat bran and wheat straw: optimization of  $\frac{475}{2}$ culture condition by Taguchi method, Enzyme Microb. Technol. 40 (2007)  $475$ <br>801–805, doi:<http://dx.doi.org/10.1016/j.enzmictec.2006.06.013>.  $477$

<span id="page-10-0"></span>10 A. Abdella et al. / Biotechnology Reports xxx (2019) e00457

- [5] S. Nagar, A. Mittal, V.K. Gupta, Enzymatic clarification of fruit juices (apple, <sup>478</sup> pineapple, and tomato) using purified Bacillus pumilus SV-85S xylanase, <sup>479</sup> Biotechnol. Bioprocess Eng. <sup>17</sup> (2012) <sup>1165</sup>–1175.
- [6] T. Periasamy, K. Aiyasamy, M.R.F.M. George, M.R.F.M. George, T. Rathinavel, M. 480 Ramasamy, Optimization of xylanase production from Aspergillus flavus in<br>481 481 solid state fermentation using agricultural waste as a substrate, Int. J. Adv.<br>482 482 Interdiscip. Res. 4 (2017) 29, doi:<http://dx.doi.org/10.29191/ijaidr.2017.4.3.06>.
- [7] H.Y. Wang, B.Q. Fan, C.H. Li, S. Liu, M. Li, Effects of rhamnolipid on the cellulase 483<br>
484 and xylanase in hydrolysis of wheat straw, Bioresour. Technol. 102 (2011)<br>
484 6515–6521.
- [8] F. Garcia-Ochoa, E. Gomez, Bioreactor scale-up and oxygen transfer rate in <sup>485</sup> microbial processes: An overview, Biotechnol. Adv. <sup>27</sup> (2009) <sup>153</sup>–176.
- [9] Y. Zhou, L.R. Han, H.W. He, B. Sang, D.L. Yu, J.T. Feng, X. Zhang, Effects of 486 agitation, aeration and temperature on production of a novel glycoprotein GP-<br>at the Character on production of a novel glycoprotein GP-<br>AB7 <sup>487</sup> <sup>1</sup> by Streptomyces kanasenisi ZX01 and scale-Up based on volumetric oxygen <sup>488</sup> transfer coefficient, Molecules. <sup>23</sup> (2018) 125.
- [10] D. Cașcaval, A.-I. Galaction, M. Turnea, Comparative analysis of oxygen transfer  $^{10}$   $^{10$ 490 broths, J. Ind. Microbiol. Biotechnol. 38 (2011) 1449–1466, doi[:http://dx.doi.](http://dx.doi.org/10.1007/s10295-010-0930-3)<br>491 [org/10.1007/s10295-010-0930-3.](http://dx.doi.org/10.1007/s10295-010-0930-3)
- [11] F. Mantzouridou, T. Roukas, P. Kotzekidou, Effect of the aeration rate and 492 agitation speed on β-carotene production and morphology of Blakeslea<br>493 agitation speed on β-carotene production and morphology of Blakeslea<br>493 between the morphology of Blakesleam Eng 493<br>trispora in a stirred tank reactor: mathematical modeling, Biochem. Eng. J. 10<br>494 (2003) 122-125-dei-http://dv.dei.erg/10.1016/51260-702X/01200166-0 (2002) 123-135, doi[:http://dx.doi.org/10.1016/S1369-703X\(01\)00166-8.](http://dx.doi.org/10.1016/S1369-703X(01)00166-8)<br>[12] J. Velasco, B. Oliva, E.J. Mulinari, L.P. Quintero, A. da Silva Lima, A.L. Gonçalves,
- [12] J. Velasco, B. Oliva, E.J. Mulinari, L.P. Quintero, A. da Silva Lima, A.L. Gonçalves, <sup>495</sup> T.A. Gonçalves, A. Damasio, F.M. Squina, A.M. Ferreira Milagres, A. Abdella, M.R. 496 Wilkins, F. Segato, Heterologous expression and functional characterization of<br>497 a GH10 endoxylanase from Aspergillus fumigatus var. Niveus with potential 498 a GH10 chuoxylanase from Aspergillus fumigatus var. Niveus with potential biotechnological application, Biotechnol. Rep. Amst. (Amst) 24 (2019) e00382, 499 doi:<http://dx.doi.org/10.1016/j.btre.2019.e00382>.
- [13] A. Abdella, F. Segato, M.R. Wilkins, Optimization of nutrient medium 500<br>  $\frac{1}{2}$ <br>  $\frac{1}{2$
- [14] A.R. De Lima, T.M. Silva, B. Fausto, R. Almeida, F.M. Squina, D.A. Ribeiro, A.F.  $[14]$  A.K. De Linia, I.M. Silva, B. Fausto, K. Alliedia, F.M. Squina, D.A. Kibelio, A.F.<br>
Paes, F. Segato, R.A. Prade, J.A. Jorge, H.F. Terenzi, M. De Lourdes, T.M. Polizeli,<br>
Heterologous expression of an Aspergillus ni
- [15] M.S. Lima, A.R.D.L. Damasio, P.M. Crnkovic, M.R. Pinto, A.M. Silva, J.C.R. Silva, F. 508 Segato, R.C. De Lucas, J.A. Demissio, P. M. Polizeli, Co-cultivation of Segato, R.C. De Lucas, J.A. Jorge, M.D.L.T.D.M. Polizeli, Co-cultivation of Segato, R. De Lucas, J.A. Despite the procession of the procession of 509 Aspergillus nidulans recombinant strains produces an enzymatic cocktail as<br>510 alternative to alkaline sugarcane bagasse pretreatment, Front Microbiol. 7<br>600 alternative to alternative to alternative pretreatment, Fron <sup>511</sup> (2016) <sup>1</sup>–9, doi:<http://dx.doi.org/10.3389/fmicb.2016.00583>.
- [16] N. Arifin, A. Lan, A.R.M. Yahya, R. Noordin, Purification of BmR1 recombinant 512 protein, Protein J. 29 (2010), doi[:http://dx.doi.org/10.1007/s10930-010-9281-](http://dx.doi.org/10.1007/s10930-010-9281-1)<br> [1.](http://dx.doi.org/10.1007/s10930-010-9281-1) [17] protein J. 29 (2010), doi:http://dx.doi.org/10.1007/s10930-010-9281-<br>
1. [17] pring, M.J. Gao, G.L. Hou, K.X. Liang, R.S. Yu, Z. Li
- $\begin{bmatrix} 11 \\ 17 \end{bmatrix}$ , Ding, M.J. Gao, G.L. Hou, K.X. Lang, R.S. Yu, Z. Li, Z.I. Shi, Stabilizing portion by Pichia pastoris with an ethanol on-line<br>515 interferon-alpha production by Pichia pastoris with an ethanol on-li 515 measurement based DO-Stat glycerol feeding strategy, J. Chem. Technol.<br>516 metashad 80.(2014) 1048-1052 <sup>516</sup> Biotechnol. <sup>89</sup> (2014) <sup>1948</sup>–1953.
- [18] K. Markošová, L. Weignerová, M. Rosenberg, V. Křen, M. Rebroš, Upscale of <sup>517</sup> recombinant <sup>α</sup>-L-rhamnosidase production by Pichia pastoris Mut(S) strain, <sup>518</sup> Front. Microbiol. <sup>6</sup> (2015) 1140, doi[:http://dx.doi.org/10.3389/](http://dx.doi.org/10.3389/fmicb.2015.01140) <sup>519</sup> [fmicb.2015.01140.](http://dx.doi.org/10.3389/fmicb.2015.01140)
- [19] T.T. Shang, D.Y. Si, D.Y. Zhang, X.H. Liu, L.M. Zhao, C. Hu, Y. Fu, R.J. Zhang,<br>
520 Enhancement of thermoalkaliphilic xylanase production by Pichia pastoris<br>
through novel fed-batch strategy in high cell-density ferm 522 Biotechnol. 17 (2017) 55.
- [20] M.L. Shuler, F. Kargi, M. DeLisa, Bioprocess Engineering: Basic Concepts, 3rd 523 edition, Prentice Hall, Boston, 2017.
- [21] A. Abdella, T.E. Mazeed, A.F. El-Baz, S.T. Yang, Production of beta-glucosidase<br>from wheat bran and glycerol by Aspergillus niger in stirred tank and rotating<br>fibrous bed bioreactors, Process Biochem. 51 (2016) 1331–1
- [22] F. Segato, A.R.L. Damasio, T.A. Goncalves, R.C. de Lucas, F.M. Squina, S.R. Decker, <sup>526</sup> R.A. Prade, High-yield secretion of multiple client proteins in Aspergillus, <sup>527</sup> Enzyme Microb. Technol. <sup>51</sup> (2012) <sup>100</sup>–106.
- [23] B. Couger, T. Weirick, A.R.L. Damasio, F. Segato, M.D.T.D. Polizeli, R.S.C. de <sup>528</sup> Almeida, G.H. Goldman, R.A. Prade, The genome of <sup>a</sup> thermo tolerant, <sup>529</sup> pathogenic albino Aspergillus fumigatus, Front. Microbiol. <sup>9</sup> (2018) 1827.
	- [24] A. Ricardo, D.L. Damasio, M.V. Rubio, T.A. Gonçalves, G.F. Persinoti, F. Segato, R.
- <sup>530</sup> A. Prade, F.J. Contesini, A.P. De Souza, M.S. Buckeridge, F.M. Squina, Xyloglucan <sup>531</sup> Breakdown by Endo-xyloglucanase Family <sup>74</sup> From Aspergillus fumigatus, <sup>532</sup> Appl. Mirobiol. Biotechnol. <sup>98</sup> (2017) <sup>2893</sup>–2903.
- [25] A. Abdella, T.E.-S. Mazeed, S.-T. Yang, A.F. El-Baz, Production of β-glucosidase by<br>
aspergillus niger on wheat bran and glycerol in submerged culture: factorial<br>
534 aspergillus niger on wheat bran and glycerol in experimental design and process optimization, Curr. Biotechnol. 3 (2014) 197–206.
- [26] A. Ferreira, G. Pereira, J.A. Teixeira, F. Rocha, Statistical tool combined with  $\frac{120 \text{ J}}{100 \text{ s}}$  analysis to characterize hydrodynamics and mass transfer in a bubble<br>state in a bubble<br>column Chem For  $\frac{130(2012) \text{ J}}{100 \text{ s}}$ <sup>536</sup> column, Chem. Eng. J. <sup>180</sup> (2012) <sup>216</sup>–228.
- [27] G.L. Miller, Use of dinitrosalicylic acid reagent for determination of reducing <sup>537</sup> sugar, Anal. Chem. <sup>31</sup> (1959) <sup>426</sup>–428.
- [28] M.M. Bradford, Rapid and sensitive method for quantitation of microgram<br>538 quantities of protein utilizing principle of protein-dye binding, Anal. Biochem.<br>539 and the protein-dye of protein-dye binding, Anal. Bioche <sup>539</sup> <sup>72</sup> (1976) <sup>248</sup>–254.
- [29] A. Sluiter, B. Hames, R. Ruiz, C. Scarlata, J. Sluiter, D. Templeton, Determination 540 of sugars, byproducts, and degradation products in liquid fraction process 540 of sugars, byproducts, and degradation products in liquid fraction process 541 samples, Golden Natl. Renew. Energy Lab. 11 (2006). 541 A.
- [30] A. Abdella, T.E.-S. Mazeed, S.-T. Yang, A.F. El-Baz, Production of <sup>β</sup>-glucosidase <sup>542</sup> by Aspergillus niger on wheat bran and glycerol in submerged culture: factorial experimental design and process optimization, Curr. Biotechnol. 3  $\frac{543}{544}$ (2014) 197–206.<br>J. Gomes, H. Purkarthofer, M. Hayn, J. Kapplmüller, M. Sinner, W. Steiner,
- [31] J. Gomes, H. Purkarthofer, M. Hayn, J. Kapplmüller, M. Sinner, W. Steiner,<br>Production of a high level of cellulase-free xylanase by the thermophilic<br>fungus Thermomyces lanuginosus in laboratory and pilot scales using<br> 548 [http://dx.doi.org/10.1007/bf00164453.](http://dx.doi.org/10.1007/bf00164453)
- [32] G. Ghoshal, U.C. Banerjee, U.S. Shivhare, Xylanase production by Penicillium 549 citrinum in laboratory-scale stirred tank reactor, Chem. Biochem. Eng. Q. 28 <sup>550</sup> (2014) <sup>399</sup>–408.
- [33] J. Sinha, J.T. Bae, J.P. Park, K.H. Kim, C.H. Song, J.W. Yun, Changes in morphology<br>of Paecilomyces japonica and their effect on broth rheology during production<br>of exo-biopolymers, Appl. Microbiol. Biotechnol. 56 (20
- [34] H. El Enshasy, E. Abuoul, Y. Helmy, Azaly, optimization of the industrial  $553$  production of alkaline protease by Bacillus licheniformis in different  $553$ <br>production of alkaline protease by Bacillus licheniformis in different  $554$ production scales, Aust. J. Basic Appl. Sci. 2 (2008) 583-593
- [35] M. Michelin, A.M. de Oliveira Mota, M. de L.T. de M. Polizeli, D.P. da Silva, A.A. For Vicente, J.A. Teixeira, Influence of volumetric oxygen transfer coefficient (kLa)<br>Uicente, J.A. Teixeira, Influence of volumetric oxygen transfer coefficient (kLa)<br>556<br>the start production by Aspergillus niger van Tie tank and internal-loop airlift bioreactors, Biochem. Eng. J. 80 (2013) 19–26, doi:<http://dx.doi.org/10.1016/j.bej.2013.09.002>. 558 [36] R. Potumarthi, S. Ch, A. Jetty, Alkaline protease production by submerged
- Formentation in stirred tank reactor using Bacillus licheniformis NCIM-2042:<br>
fermentation in stirred tank reactor using Bacillus licheniformis NCIM-2042:<br>
560 650 effect of aeration and agitation regimes, Biochem. Eng. J. 34 (2007) 185–192,  $561$  doi:<http://dx.doi.org/10.1016/j.bej.2006.12.003>.
- [37] M.S. Bhattacharyya, A. Singh, U.C. Banerjee, Production of carbonyl reductase 6.5. biatracharyya, A. Shigh, O.C. bancijec, Froduction of carbonyi reductase<br>by Geotrichum candidum in a laboratory scale bioreactor, Bioresour. Technol.<br>563 <sup>563</sup> <sup>99</sup> (2008) <sup>8765</sup>–8770.
- [38] R.R. Singhania, R.K. Sukumaran, K.P. Rajasree, A. Mathew, L. Gottumukkala, A. Fandey, Properties of a major beta-glucosidase-BGL1 from Aspergillus niger<br>Pandey, Properties of a major beta-glucosidase-BGL1 from Aspergillus niger<br>NII-08121 expressed differentially in response to carbon sources, Proces <sup>566</sup> Biochem. <sup>46</sup> (2011) <sup>1521</sup>–1524.
- [39] J.F. de Burkert, R.R. Maldonado, F. Maugeri Filho, M.I. Rodrigues, Comparison of 567 chem. Technol. Biotechnol. 80 (2005) 61–67, doi[:http://dx.doi.org/10.1002/](http://dx.doi.org/10.1002/jctb.1157)<br>Chem. Technol. Biotechnol. 80 (2005) 61–67, doi:http://dx.doi.org/10.1002/<br>169 sictb.1157.<br>169 sictb.1157.
- [40] C. Bandaiphet, P. Prasertsan, Effect of aeration and agitation rates and scale-up <sup>570</sup> on oxygen transfer coefficient, k(L)a in exopolysaccharide production from Enterobacter cloacae WD7, Carbohydr. Polym. 66 (2006) 216–228.<br>[41] M. Fenice, P. Barghini, L. Selbmann, F. Federici, Combined effects of agitation
- M. Fence, F. Barginin, E. Selbmann, F. Federici, Combined effects of agrication of the chitrinolytic enzymes production by the Antarctic fungus 572<br>and aeration on the chitrinolytic enzymes production by the Antarctic fung
- [42] L. Dos Reis, R.C. Fontana, P. da Silva Delabona, D.J. da Silva Lima, M. Camassola,<br>J.G. da Cruz Pradella, A.J.P. Dillon, Increased production of cellulases and<br>xylanases by Penicillium echinulatum S1M29 in batch and f 576 Bioresour. Technol. 146 (2013) 597–603, doi:[http://dx.doi.org/10.1016/j.](http://dx.doi.org/10.1016/j.biortech.2013.07.124)<br>Bioresour. Technol. 146 (2013) 597–603, doi:http://dx.doi.org/10.1016/j. 577<br>[43] M. Muller, F. Segato, R.A. Prade, M.R. Wilkins, High-yield reco
- [43] M. Muller, F. Segato, R.A. Prade, M.R. Wilkins, High-yield recombinant xylanase <sup>578</sup> production by Aspergillus nidulans under pyridoxine limitation, J. Ind.
- Microbiol. Biotechnol. 41 (2014) 1563–1570.<br>R.S.S. Teixeira, F.G. Siqueira, M.V. de Souza, E.X. Ferreira Filho, E.P. da Silva Bon, [44] R.S.S. Teixeira, F.G. Siqueira, M.V. de Souza, E.X. Ferreira Filho, E.P. da Silva Bon, <sup>580</sup> Purification and characterization studies of <sup>a</sup> thermostable <sup>β</sup>-xylanase from <sup>581</sup> Aspergillus awamori, J. Ind. Microbiol. Biotechnol. <sup>37</sup> (2010) <sup>1041</sup>–1051.
- [45] C. Techapun, N. Poosaran, M. Watanabe, K. Sasaki, Optimization of aeration and E. Fechapun, N. Poosaran, M. Vadanabe, N. Sasaki, Ophilization of actation and<br>agitation rates to improve cellulase-free xylanase production by<br>thermotolerant Streptomyces sp. Ab106 and repeated fed-batch cultivation<br>584<br>5 <sup>584</sup> using agricultural waste, J. Biosci. Bioeng. <sup>95</sup> (2003) <sup>298</sup>–301, doi[:http://dx.](http://dx.doi.org/10.1016/S1389-1723(03)80033-6) <sup>585</sup> [doi.org/10.1016/S1389-1723\(03\)80033-6](http://dx.doi.org/10.1016/S1389-1723(03)80033-6).
- [46] R. Bandikari, U. Katike, N.S. Seelam, V.S.R. Obulam, Valorization of de-oiled 586 cakes for xylanase production and optimization using central composite 586<br>design by Trichoderma koeningi isolate, Turkish J. Biochem. Biyokim. Derg. 42 <sup>588</sup> (2017) <sup>317</sup>–328.
- [47] H. Moteshafi, S.M. Mousavi, M. Hashemi, Enhancement of xylanase<br>productivity using industrial by-products under solid suspended<br>fermentation in a stirred tank bioreactor, with a dissolved oxygen constant<br>control stra
- [48] Y. Bakri, M. Al-Jazairi, G. Al-Kayat, Xylanase production by a newly isolated <sup>593</sup> Aspergillus niger SS7 in submerged culture, Polish J. Microbiol. <sup>57</sup> (2008) <sup>249</sup>– <sup>594</sup> 251.
- [49] Y. Cao, D.J. Meng, J. Lu, J. Long, Statistical optimization of xylanase production<br>by Aspergillus niger AN-13 under submerged fermentation using response<br>surface methodology, African J. Biotechnol. 7 (2008) 631–638. 5
- [50] Y. Bakri, A. Mekaeel, A. Koreih, Influence of agitation speeds and aeration rates <sup>597</sup> on the Xylanase activity of Aspergillus niger SS7, Braz. Arch. Biol. Technol. <sup>54</sup> <sup>598</sup> (2011) <sup>659</sup>–664, doi:<http://dx.doi.org/10.1590/s1516-89132011000400003>.
- [51] S.W. Kim, S.W. Kang, J.S. Lee, Cellulase and xylanase production by Aspergillus <sup>599</sup> niger KKS in various bioreactors, Bioresour. Technol. <sup>59</sup> (1997) <sup>63</sup>–67, doi: [http://dx.doi.org/10.1016/S0960-8524\(96\)00127-7.](http://dx.doi.org/10.1016/S0960-8524(96)00127-7)