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# Improvement of Oxygen Transfer Coefficient During *Penicillium canescens* Culture

## Influence of Turbine Design, Agitation Speed, and Air Flow Rate on Xylanase Production

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## ABSTRACT

To improve xylanase productivity from *Penicillium canescens 10–10c* culture, an optimization of oxygen supply is required. Because the strain is sensitive to shear forces, leading to lower xylanase productivity as to morphological alteration, vigorous mixing is not desired. The influence of turbine design, agitation speed, and air flow rate on $K_La$ (global mass transfer coefficient, $h^{-1}$) and enzyme production is discussed. $K_La$ values increased with agitation speed and air flow rate, whatever the impeller, in our assay conditions. Agitation had more influence on $K_La$ values than air flow, when a disk-mounted blade's impeller (DT) is used; an opposite result was obtained with a hub-mounted pitched blade's impeller (PBT). Xylanase production appeared as a function of specific power ($W/m^3$), and an optimum was found in 20 and 100 L STRs fitted with DT impellers. On the other hand, the use of a hub-mounted pitched blade impeller (PBT8), instead of a disk-mounted blade impeller (DT4), reduced the lag time of hemicellulase production and increased xylanase productivity 1.3-fold.

**Index Entries:** Xylanase; *Penicillium canescens*; $K_La$; specific power.

## INTRODUCTION

Aerobic microorganism growth requires a continuous and efficient supply of oxygen, and hence effective gas–liquid mass transfer. This is traditionally realized by gas-sparged stirred-tank reactor *(1)*. The process of making oxygen available to a growing culture is a critical factor in

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aerobic cultivation, since agitation could create high shear environment *(2–4)*; that may lead to mechanical disruption of cells, and hence impaired reactor performance. Examples of shear effects on biomass and product formations are abundant *(2,5–11)*, and many authors showed a dependence of cellulase and xylanase production on agitation or aeration rate *(10,12–15)*. Nevertheless, dependence of product formation with scaling-up parameters, such as specific power, peripheral tip speed, gas hold-up, or circulation time, are less common.

In this work, the xylanase production by *P. canescens 10–10c* was studied; earlier results showed the dependence of hyphal growth on agitation speed and oxygen supply. This suggested investigation of the influence of turbine design, agitation speed, and aeration rate on $K_la$ in reactors fitted with one impeller. Results on $K_la$, specific power, and production in mono-agitated tanks are discussed.

## MATERIALS AND METHODS

### Strain

*P. canescens 10–10c* was provided by G. I. Kvesidatse, Institute of Plant Biochemistry, Academy of Sciences, Tbilisi, Georgia.

### Culture Medium

The culture medium contained 30 g/L wheat straw, 30 g/L soya meal, and 5 g/L yeast extract, in mineral salt medium. The mineral salt medium contained: $Na_2HPO_4{\cdot}7H_2O$ (1.5% w/v), KCl (0.05% w/v), $MgSO_4$ 7 $H_2O$ (0.015% w/v).

### Stirred-Tank Reactor (STR) (Diagram 1)

Fermentations were carried out in Biolafitte 20-L stirred-tank reactor (STR) fitted either with Rushton disk turbines with 6 or 4 blades (DT6, DT4, respectively), or pitched blade turbines with 8 or 4 blades (PBT8, PBT4, respectively). Turbine diameter (d) was 0.1 m. The working volume was 8.5 L, to have liquid height (H) equal to STR diameter (D = 0.23 m). Turbine was placed at height (h) D/3 from the bottom of the reactor. Four baffles were 0.49 m in height and 0.032 m in width. STRs were inoculated to give a spore concentration of $10^6$ sp/mL, and temperature was maintained at 30°C. pH was not controlled, and varied between 5.8 and 7.5.

### $K_la$ Measurements

$K_la$ was calculated with the static gassing-out method, in the presence of the mycelium killed by addition of sodium aside (0.5 g/L) followed by a thermal treatment at 60°C for 30 min. This procedure permitted work at

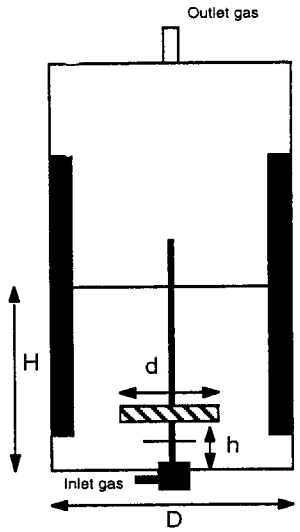


Diagram 1. Description of the STR.

real rheological culture conditions. All measurements were realized at 30°C and pH 7.0.

## Xylanase Assay

Xylanase assay was carried out according to Bailey *(16)*. One unit (U) of enzyme activity is defined as the amount of sugar (in μmol) produced per min of reaction and per mL of enzyme solution, in the assay conditions.

## Oxygen and Carbon Dioxide Analyzers

Oxygen and carbon dioxide were analyzed on-line with, respectively, Servomex OA 570 (Crowborough, England) and Binos (Leybold-Herous, Hanau, Germany) analyzers. This permitted the calculation of the $K_l a$ (on-line), the respiratory quotient (on-line), the oxygen uptake rate (OUR) and the carbon dioxide transfer rate (CTR).

## Specific Power Calculation

The specific power ($W/m^3$) was calculated with the Calderbank relation *(17)*:

$$P = (1 - 12{,}6\ G/Nd^3)\ (Np\ \rho\ N^3\ d^5)\ (1/V)$$

where V is the culture volume ($m^3$), N is the agitation speed ($h^{-1}$), d is the impeller diameter (m), ρ is the volumic mass ($kg/m^3$) of the medium, G is the aeration rate ($m^3/h$) and Np is the power number.

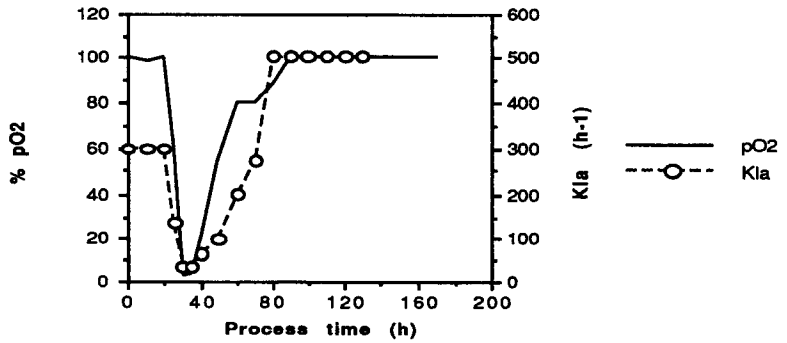


Fig. 1. $pO_2$ and $K_la$ (global transfer coefficient $h^{-1}$) evolution during growth of *P. canescens 10–10c* in a 20-L STR on wheat straw (3% w/v), soya meal (3% w/v), yeast extract (0.5% w/v), in mineral salt medium.

## RESULTS

### Evolution of $pO_2$ and $K_la$ During *P. canescens* Culture

Figure 1 shows the time-course of $pO_2$ and $K_la$ evolution during a cultivation of *P. canescens* on wheat straw and soya meal medium. Aeration rate was 0.75 vvm, agitation rate was 300 rpm, and the STR was fitted with one DT4.

During growth, $pO_2$ decreased to 0% of saturation for several hours, and $K_la$ decreased from 60 $h^{-1}$ to 37 $h^{-1}$; this could result from the increase in broth viscosity. Xylanase production started after the growth phase (after about 40 h of culture time). These results clearly indicated that there is a lack in oxygen supply, and that the culture needs an improvement of the oxygen transfer coefficient ($K_la$), which could lead to enhanced biomass production, and hence product formation.

Hereunder are presented results on the influence of factors acting on $K_la$ in STR fitted with one or two impellers. Dependence of xylanase production on $K_la$ and specific power in mono-agitated STR are also shown.

### Improvement of Oxygen Transfer in *P. canescens* 10-10c Culture: Influence of Agitation and Aeration Rate on $K_la$ in Mono-agitated Reactor

The influence of agitation speed and air flow rate on $K_la$ in *P. canescens* culture was evaluated; the parameters studied were the turbine (DT4, DT6, and PBT8), the air flow rate (0.3, 0.5 and 0.75 vvm), and the peripheral speed (0.78, 1.05, 1.31, and 1.57 m/s). Results are shown in Figs. 2–4.

For each experiment, $K_la$ values increased with peripheral speed; at high agitation speed (1.57 m/s), the most effective turbine for mass transfer was the DT6 one. $K_la$ values for the PBT8 showed greater dependence

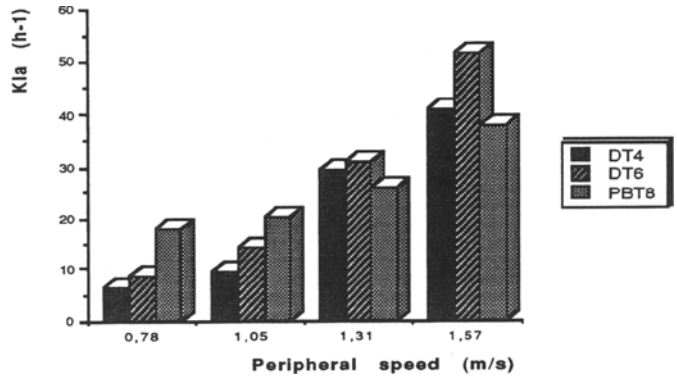


Fig. 2. $K_la$ ($h^{-1}$) as a function of peripheral speed and turbine design, in a *P. canescens* culture on soya meal and wheat straw. Aeration rate is fixed at 0.3 vvm.

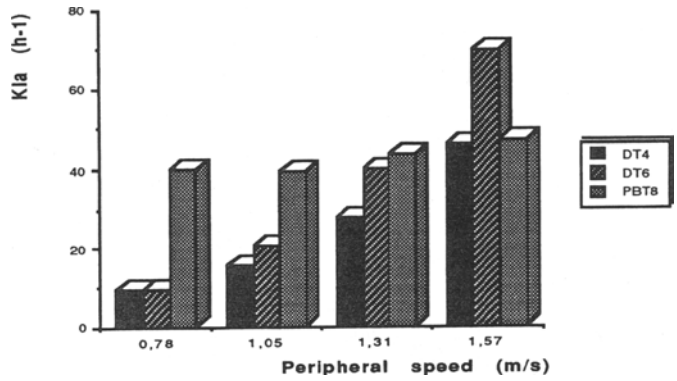


Fig. 3. $K_la$ ($h^{-1}$) as a function of peripheral speed and turbine design, in a *P. canescens* culture on soya meal and wheat straw. Aeration rate is fixed at 0.5 vvm.

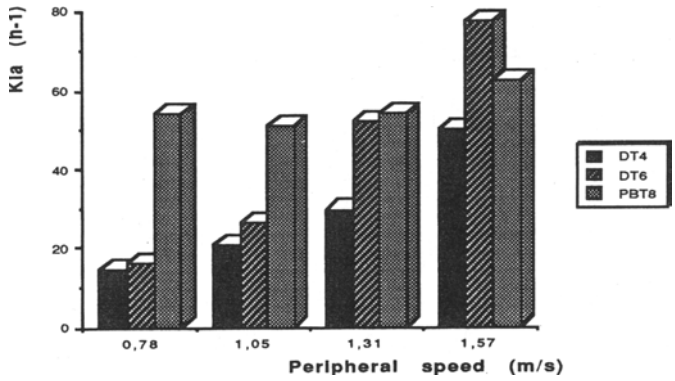


Fig. 4. $K_la$ ($h^{-1}$) as a function of peripheral speed and turbine design. Aeration rate is fixed at 0.75 vvm.

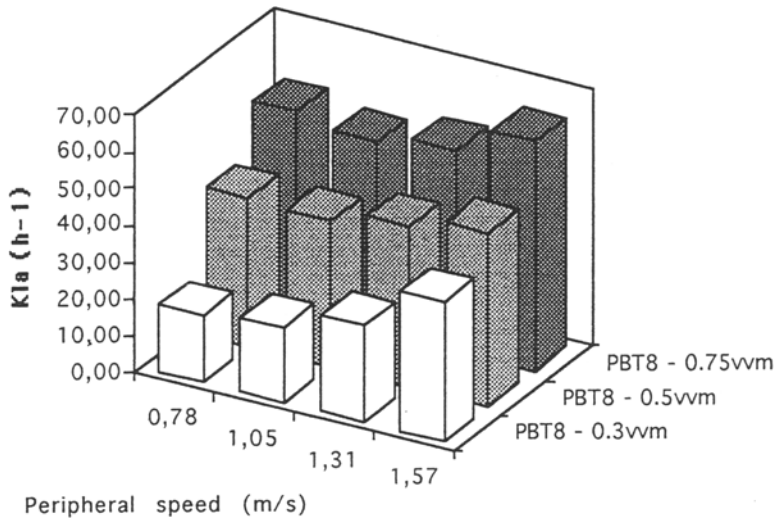


Fig. 5. $K_la$ ($h^{-1}$) as a function of peripheral speed and aeration rate for PBT8 impeller.

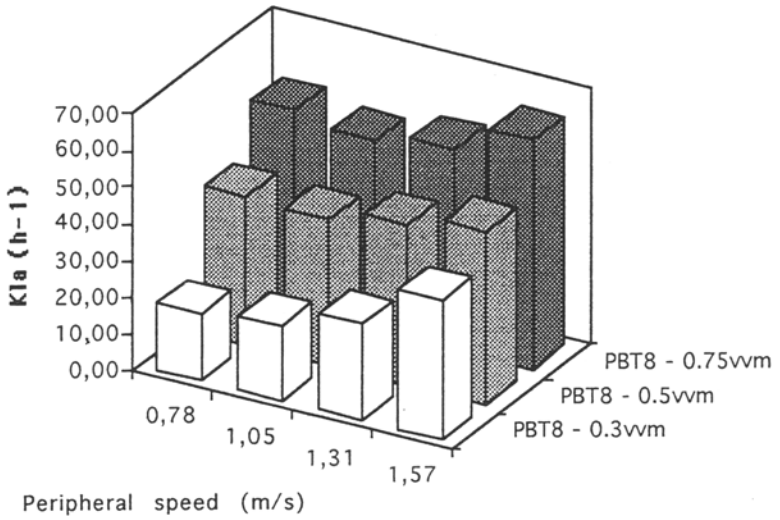


Fig. 6. $K_la$ ($h^{-1}$) as a function of peripheral speed and aeration rate for DT4 impeller.

with air flow rate than with agitation speed, unlike the DT impellers (Figs. 5 and 6). The relative efficiency of PBT8 impeller grew up when air flow rate was increased and agitation speed was reduced; this means that the ratio $K_la_{PBT8}$ on $K_la_{DT}$ was greater at lower aeration and agitation rates than at higher gassing and mixing conditions.

Figure 7 shows $K_la$ as a function of specific power P ($W/m^3$) and aeration rate G ($m^3/h$) for DT impellers (both DT6 and DT4). Concerning

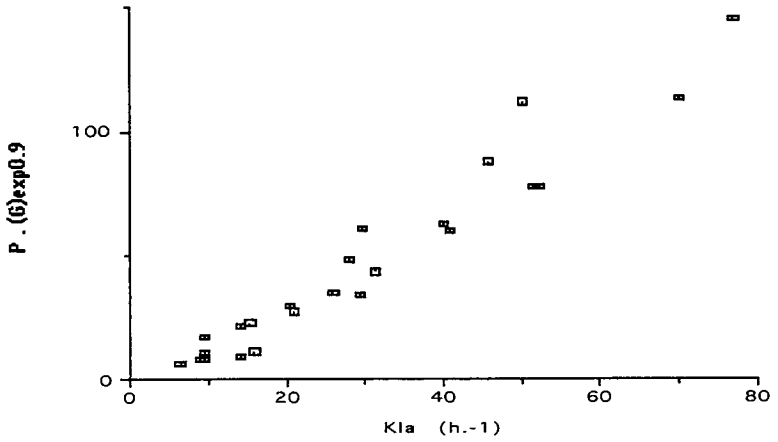


Fig. 7. $K_la$ as a function of specific power ($W/m^3$) and aeration rate ($m^3/h$). Data for DT6 and DT4 impellers.

PBT impeller, $K_la$ was a function of pumping rate Qp ($m^3/h$) and aeration rate G ($m^3/h$) (Data not shown).

## Influence of Turbine Design on Xylanase Production

At first, the influence of agitation speed on xylanase inactivation was evaluated: Results revealed that, in the presence of biomass, no xylanase denaturation occurred when agitation was enhanced (data not shown).

Xylanase production was carried out in STRs fitted with one impeller. Multiagitated aerated reactors were not yet evaluated: hydrodynamic currents in these reactors are complex, and correlations between results and scaling-up parameters are hazardous *(19–21)*. Figure 8 shows the time-course of xylanase production during batch culture of *P. canescens 10-10c* in a 20-L STR, fitted either with a PBT8 or a DT4 (all other culture parameters remaining unchanged). After 144 h of culture time, the batch culture with the PBT8 turbine showed relatively greater xylanase yield (844 U/mL) than the batch with DT4 (722 U/mL). Figure 8 also reveals that xylanase production started earlier with PBT8 impeller than with the DT4, but after that, production rates are similar in both cases.

Xylanase productivity was also improved when a PBT8 (844 U/mL) was used instead of PBT4 (683 U/mL) (all other culture conditions remaining unchanged).

Figure 9 shows that production after 140 h of culture time is a function of the specific power ($W/m^3$).

Xylanase production showed an optimum with specific power about 200–500 $W/m^3$. This was found in 20-L STR, fitted with DT6 or DT4 impeller, and in 100-L STR fitted with DT4.

At 200 rpm (102 $W/m^3$) in 20-L STR, oxygen transfer was poor, $pO_2$ was under 5% of saturation during 37 h and xylanase production reached

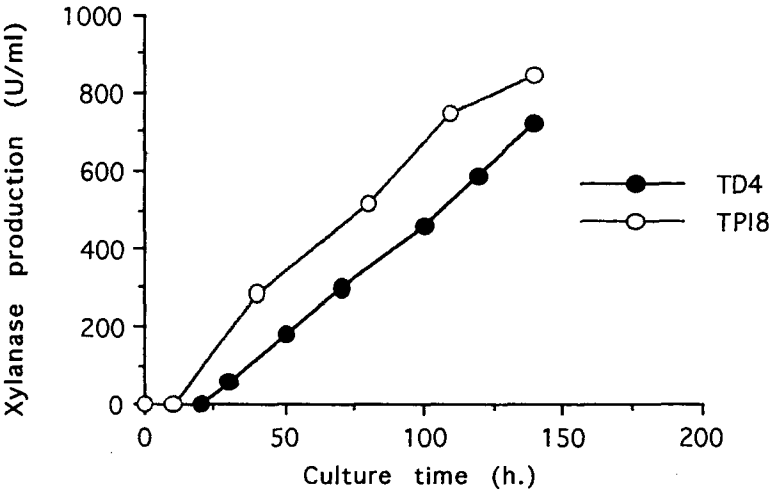


Fig. 8. Evolution of xylanase production (U/mL) during batch culture of *P. canescens 10–10c* in a 20-L STR fitted either with a hub-mounted pitched blade turbine (PBT8) or a disk-mounted blade turbine (DT4). Agitation speed: 300 rpm, aeration rate: 0.75 vvm.

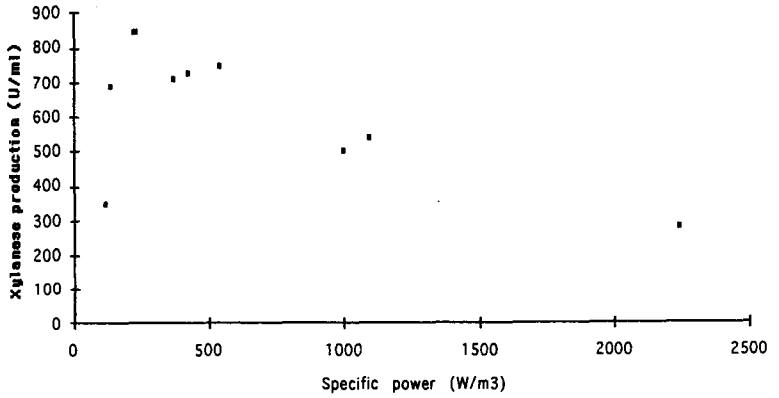


Fig. 9. Xylanase production as a function of specific power in 20-L STR. Data for DT6, DT4, PBT8, and PBT4 impellers.

a low value of 370 U/mL. On the other hand, high agitation speed produced hydrodynamic stress of hyphae; under certain conditions, hyphal break-ups were observed, and this could explain the decrease in productivity above 500 W/m$^3$. This could explain the optimum in specific power.

In 6-L STR (working volume: 2.5 $10^{-3}$ m$^3$), a continuous decrease in productivity was observed between 500 W/m$^3$ (450 rpm, DT6 diameter: 0.06 m) and 25,000 W/m$^3$ (1200 rpm, DT6 diameter: 0.07 m).

Xylanase production with PBT8 impeller reached 844 U/mL for a specific power estimated to 230 W/m$^3$ (Fig. 8).

## DISCUSSION

### Improvement of Oxygen Transfer in *P. canescens* 10-10c Culture: Influence of Agitation and Aeration Rate on $K_la$ in Monoagitated Reactor

In preliminary experiments *(22)*, the authors studied, in shake flasks and in STR, the optimization of culture conditions for biomass and xylanase production from *P. canescens 10-10c*. Results showed that good yields were obtained when mass transfer was optimized. Nevertheless, culture of many filamentous microorganisms in STR copes with a similar problem: poor oxygen transfer and hydrodynamic stress of hyphae *(8–11)*.

Growth of *P. canescens 10-10c* on wheat straw, soya meal, and yeast extract in a 20-L STR developed a high-viscosity broth: This led to a drastic decrease of $K_la$ value from 300 to $37h^{-1}$. It was concluded that an insufficient oxygen transfer limited the mycelial growth.

The influence of factors acting on $K_la$ value in real-culture conditions was investigated. The objective is to enhance oxygen transfer and to avoid hyphal stress. The influence of mobil design on $K_la$ and hemicellulase production, for different agitation and air flow rates, was evaluated. Performances of shearing and pumping turbines were compared in a specific medium containing straw, soya meal, and filamentous biomass.

For each experiment, $K_la$ increased with agitation and aeration rates, whatever the impeller. $K_la$ values were more influenced by agitation speed than by air flow rate, when DT impellers were used; an opposite result was obtained with PBT8. These observations are explained by the specific action of the two impellers, shearing (DT) or pumping (PBT8).

DT impeller, also known as radial-flow impeller *(23)*, creates shear forces; these forces, settled by peripheral tip speed and specific power, influence bubble diameter. On the other hand, PBT8 impeller is an axial-flow impeller. It creates less shear forces and induces higher circulation time of bubbles, and hence gas hold-up. All these parameters act on the mass transfer coefficient.

Results revealed that a good compromise for *P. canescens* culture could be obtained with PBT8 impeller, because such a turbine generated lower shear forces than radial turbines, at the same agitation rate, without dramatic lost in oxygen transfer capacity.

### Influence of Turbine Design on Xylanase Production

These results on $K_la$ were correlated with xylanase production in monoagitated STR. The use of PBT8 instead of DT4 reduced the lag time of xylanase production; DT4 presented an intermediate value: at 300 rpm and 0.75 vvm, xylanase production after 50 h of culture reached, respectively, 300 U/mL with a PBT8, 150 U/mL with a DT4, and 64 U/mL with

a DT6. Subsequently, after 140 h of culture time, productivity was improved by using a PBT8, instead of a DT4, by a factor 1.3.

Two things could explain these results: first, $K_la$ values were higher with a PTB8 than with a DT4; subsequently, it could be expected to have a better biomass, and hence xylanase yields in the first case. This assumption was not verified when comparing results of DT6 and PBT8 production. Thus, another way to explain our results is the influence of stirring conditions on production: Because the specific power generated by PBT8 is lower than with DT6 impeller (all others conditions remaining unchanged), lower shear forces result from PBT8 than DT6 *(19–21)*, and, although $K_la_{DT6}$ was higher than $K_la_{PBT8}$, productivity was enhanced by using an axial impeller. The effects of high agitation speed on biomass production was already demonstrated *(22)*, and many authors reported influence of hydrodynamic stress on filamentous microorganisms *(6–9)*. From these results, it was concluded that a reduction in specific power is more profitable to xylanase production than an increase in $K_la$, beyond a critical value.

Too poor $K_la$ is harmful to biomass growth: This was verified when production at 200 rpm with DT4 ($P$ = 102 W/m$^3$, $K_la$ of about 20 h$^{-1}$) reached only 370 U/mL. An optimum was found at 300 rpm with PBT8 ($P$ = about 232 W/m$^3$, $K_la$ = about 62 h$^{-1}$), and production reached 844 U/mL.

For similar reasons, xylanase productivity was also enhanced when STR was fitted with a PBT8, instead of a PBT4; indeed, as 8 blades furnished a higher pumping rate than 4 blades, and subsequently higher $K_la$ values, productivity was improved.

Results also revealed that specific power could be useful for scaling-up the process: A value of 200–500 W/m$^3$ should be maintained to ensure best xylanase yield. Production in 500 L and 2 m$^3$ are planned, to verify this assumption.

## CONCLUSION

*P. canescens 10-10c* is subject to hydrodynamic stress, and its culture in STR has to resolve contradictory aims: to increase oxygen transfer without excessive mixing. The authors propose to use a pitched blades turbine instead of a Rushton turbine; this solution permitted increased xylanase production because of lower shear forces induced. However, solutions have to be found that combine low shear and high oxygen transfer rate. Air-lift reactors could be investigated in this way.

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