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Influence of a New Axial Impeller on K_La and Xylanase Production by *Penicillium canescens* 10-10c

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Abstract

The effects of a new axial impeller (HTPG4) on oxygen volumetric transfer coefficient, K_La, and xylanase production by *Penicillium canescens* 10-10c were studied and compared for dual-impeller systems, one with one DT4 impeller below and one HTPG4 above (DT4-HTPG4) and one with two DT4 (DT4-DT4) impellers, in a 5-L bioreactor. The volumetric coefficient of oxygen transfer was measured in culture medium using a gassing-out method at different gassing rates and agitation speeds. We observed that the DT4-HTPG4 combination provided better K_La performance than the DT4-DT4 combination. The two combinations were also tested for their influence on xylanase production by a filamentous microorganism; *P. canescens* 10-10c. These experiments demonstrated that the DT4-HTPG4 combination impeller enhanced enzyme production up to 23% compared with the DT4-DT4 combination at an aeration rate of 1 vvm and an agitation speed of 600 rpm. The main cause for this difference is thought to be a higher shear stress generated by the DT4-DT4 combination, which damages the mycelium of *P. canescens* and decreases xylanase production.

Index Entries: K_La; xylanase; *Penicillium canescens*; down-pumping impeller; dual-impeller.

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Introduction

Oxygen transfer rate is often the limiting factor for bioprocesses. When aerobic processes are carried out in aqueous medium with ionic salts, the rate of oxygen transfer is limited owing to the low solubility of oxygen. The rate of oxygen utilization by microorganisms for growth and activity is considerably high, and any shortage of oxygen drastically affects the process performance *(1)*. However, the efficient breakup of gas bubbles for conventional oxygenation cannot be directly used in shear-sensitive biologic systems *(2)*. Therefore, the design of bioreactors which gives the maximum potential of microorganisms productivity and product concentration achievable is one of the most challenging tasks in the biochemical industry. In such processes, the availability of oxygen depends on mixing and mass transfer, which, in turn, are primarily governed by vessel design, type of impeller, and fluid properties *(3)*. Thus, the choice and design of the impeller are critical for maintaining proper hydrodynamic conditions and gas-liquid mass transfer rates in the reactor.

Traditionally, the radial flow disk turbines, typically the Rushton disk turbine with a diameter (d) one third of the reactor diameter (D), are the most commonly used mixers and are considered to be good for gas dispersion in chemical industry processes *(4)*. However, disk turbines have several disadvantages. For example, the power numbers of disk turbines are substantially higher than those of axial-flow impellers and vary for certain kinds by a factor of 20, as the difference between the power number for the DT6 (5.39) and the profiled-blade axial impeller HE-3 (0.27) *(5)*. In addition, the power drawn for a radial impeller under aerated conditions falls by as much as 50–65% of its unaerated value *(6)*. Therefore, new modifications of standard impeller types arise in order to improve the performance of the systems *(7–10)*. The main aim is to improve the performance of a gas transfer, controlling the drag experienced by the impeller blades so as to reduce the power consumption, to modify the flow patterns in the reactor or to have a uniform shear distribution so that the microorganisms are unaffected.

Multiple-impeller systems are found to be more efficient in mass transfer than the single impeller. Single-impeller stirred tanks are often criticized for the uneven distribution of shear and energy dissipation, which are known to be harmful, especially to the microorganisms in the bioreactors. For equivalent power dissipation, impeller speeds will be lower in the case of multiple-impeller systems, resulting in lower values of maximum shear generated. Note that the shear forces related to contributions from bubble bursting at the interface will remain the same in single- and multiple-impeller systems, and, hence, the overall cell destruction rate resulting from fluid shear is expected to be lower in the multiple-impeller systems dissipating the same overall power. Thus, multiple-impeller systems will be favored when shear sensitivity to microorganisms is an important criteria for design *(1)*.

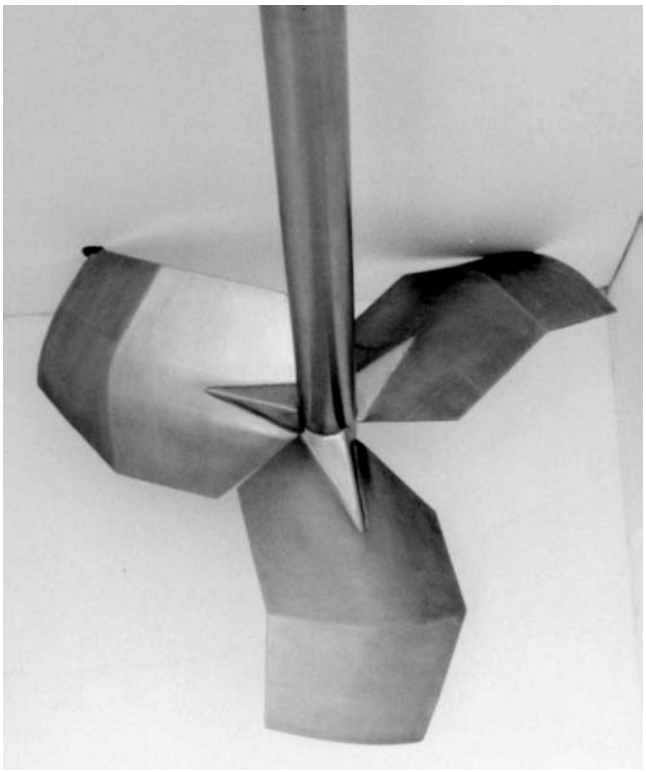

Fig. 1. HTPG4 impeller.

The production of cellulase and xylanase by *Aspergillus fumigatus*, *Aspergillus wentii*, *Trichoderma reesei* and *Penicillium janthinellum* has been shown to be influenced by stirring rates *(11–13)*. Previous studies in our laboratory using a monoagitator bioreactor have demonstrated that xylanase production by *Penicillium canescens* was influenced by the type of impeller *(14,15)*. The studies showed that the DT impeller creates important shear forces causing hyphal breakup and decreased xylanase production compared with an axial impeller PBT8, which creates less shear forces and induces higher xylanase production. We, therefore recommended an improved oxygen transfer without excessive shear for the hyphae by modifying the turbine design to reduce shear stress without loss of oxygen transfer capacity.

The aim of the present work was to study the influence of a new axial impeller, HTPG4 (Fig. 1), on volumetric oxygen transfer coefficient, $K_L a$, and xylanase production by *P. canescens*. The HTPG4 impeller was designed by Pierre Guerin SA (Mauze-sur-le-mignon, France) and has a low power number (0.67). The influence of two combinations of impellers, two radial impellers DT4 that have a high power number (3.42) *(5)* on one side, and one DT4 below and one HTPG4 above on the other side, were first

tested for their influence on K_La values at different aeration rates and agitation speeds. These two combinations were also used in cultures of *P. canescens* 10-10c, and their influence on xylanase production by this strain was evaluated.

Materials and Methods

Strain

P. canescens 10-10c was supplied by G. I. Kvesidatse, Institute of Plant Biochemistry, Academy of Sciences, Tbilisi, Georgia.

Culture Medium

The culture medium used for studying biomass production contained glucose (2%), peptone (1%), and yeast extract (1%). The culture medium used for K_La measurements and xylanase production contained 10 g/L of wheat bran and 5 g/L of yeast extract, in mineral salt medium. The mineral salt medium contained $(NH_4)_2SO_4$ (0.2% w/v), $Na_2HPO_4{\cdot}2H_2O$ (1% w/v), KCl (0.05% w/v), and $MgSO_4{\cdot}7H_2O$ (0.015% w/v). Tego antifoam KS911 (0.05% [v/v]; Goldschmidt, Essen, Germany) was added to each culture medium in the bioreactor.

Bioreactor

A 5-L Biolafitte bioreactor (Pierre Guerin SA) fitted with dual impellers was employed as the base mechanical vessel, with two Rushton disk turbines DT4 or one Rushton disk turbine DT4 in the lower position and one new impeller HTPG4 in the upper position. Both impellers have a diameter (d) of one third of the bioreactor diameter (D), and the bioreactor volume used had an H/D = 1.15. The clearance above the base of the bioreactor and the distance between the two impellers were equal to d. The bioreactor was filled with 3.8 L of production medium and then sterilized at 121°C for 30 min. A regulation system was used to control the temperature at 30°C throughout the experiment after sterilization. The bioreactor was inoculated with a 5% vol of inoculum under aseptic conditions. The inoculum was prepared in a 500-mL Erlenmeyer flask in the medium containing 2% glucose, 1% yeast extract, and 1% peptone. The inoculum medium was inoculated after sterilization with a spore suspension to give a spore concentration of 10^6 spores/mL and incubated at 30°C under shaking (125 rpm) for 24 h.

Measurement of K_La

In the absence of microorganism, the K_La was determined by the gassing-out method according to Ozbek and Gayik *(16)*. The airflow rates studied were 0.5, 0.75, and 1 vvm at agitation speeds of 300, 450, 600, 750, and 900 rpm corresponding to the following peripheral speeds of 0.91, 1.37, 1.82, 2.28, and 2.74 m/s, respectively. During the culture of *P. canescens*, the

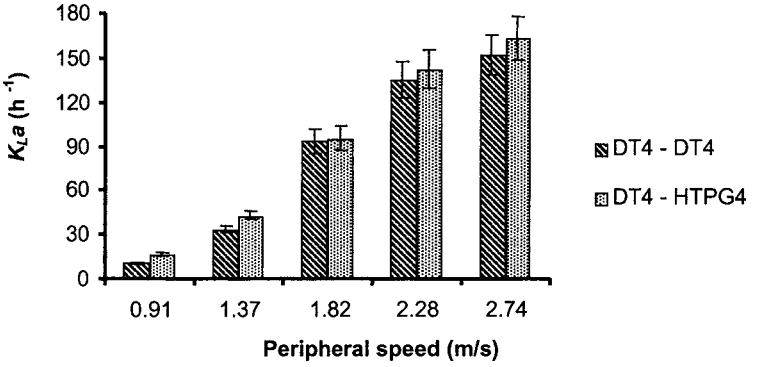

Fig. 2. $K_L a$ (h^{-1}) as a function of peripheral speed and dual-impeller design in culture medium. The aeration rate was fixed at 0.5 vvm.

$K_L a$ was evaluated by the gas balance technique. Oxygen and carbon dioxide were analyzed online with Servomex OA 570 analyzers (Crowborough, England). $K_L a$ values were obtained from the data using Eq. 1:

$$K_L a = \mathrm{OUR}/(C^* - C_L) \quad (1)$$

in which OUR, the oxygen uptake rate by the microorganisms, is determined as described by Schell et al. *(17)*. C^* and C_L refer to the liquid phase oxygen concentration at saturation and at any time, respectively. This last value is measured with a polarographic oxygen electrode (Knick, Berlin, Germany).

Xylanase Assay

Xylanase activity was measured according to Bailey et al. *(18)* using 1% birchwood xylan as substrate; reducing sugars were assayed by a dinitrosalicylic acid method with xylose as the standard *(19)*. One unit of enzyme activity is defined as the amount of sugar (in micromoles) produced per minute of reaction and per milliliter of enzyme solution, in the assay conditions.

Results

Influence of Dual-Impeller Design on $K_L a$

The influence of airflow rate and agitation speed on $K_L a$ was investigated at five peripheral speeds (0.91, 1.37, 1.82, 2.28, and 2.74 m/s) and three different airflow rates (0.5, 0.75, and 1 vvm). Two combinations of impellers were used, one with two DT4 (DT4-DT4) impellers and the other with one DT4 below and one HTPG4 above (DT4-HTPG4). Figs. 2–4 show that the $K_L a$ values for both impeller combinations were enhanced with an increase in agitation speed and airflow rate. At low peripheral speed (0.91 m/s), the $K_L a$ values were significantly better with DT4-HTPG4 compared with DT4-DT4. The combination DT4-HTPG4 gave $K_L a$ values 35, 44,

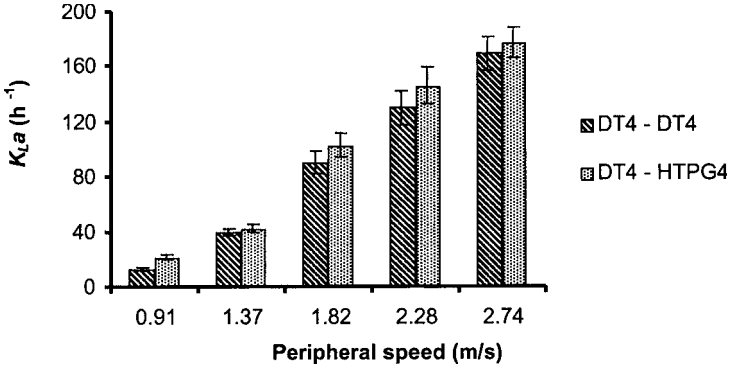

Fig. 3. K_La (h^{-1}) as a function of peripheral speed and dual-impeller design in culture medium. The aeration rate was fixed at 0.75 vvm.

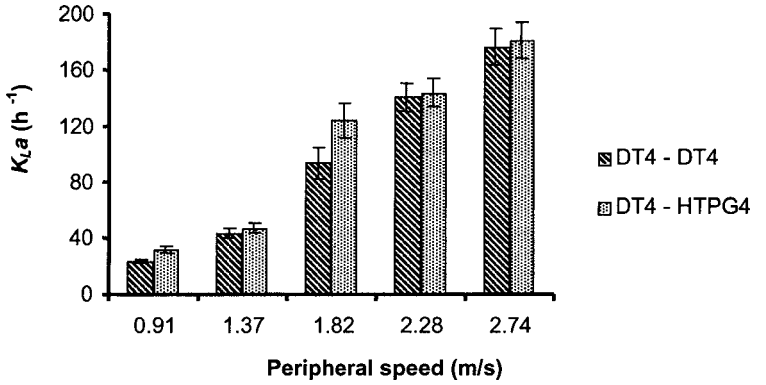

Fig. 4. K_La (h^{-1}) as a function of peripheral speed and dual-impeller design in culture medium. The aeration rate was fixed at 1 vvm.

and 25% higher than those obtained with DT4-DT4 for 0.5, 0.75, and 1 vvm, respectively. At higher peripheral speed (2.74 m/s), the differences between the two combinations were not significant.

Effect of Impeller Combination on Penicillium *Growth*

A first set of experiments was performed in a 5-L bioreactor in the presence of the two different combinations of impellers in a culture medium containing soluble substrate in order to follow biomass production and substrate consumption. No significant production of xylanase was observed in such a culture broth owing to the catabolic repression of glucose and the absence of inducer. As shown in Fig. 5, growth rate and glucose consumption were similar for the two impeller combinations. However, after 28 h of culture, a decline in biomass was observed with the DT4-DT4 combination. This could be the result of a higher shear stress generated by this impeller design.

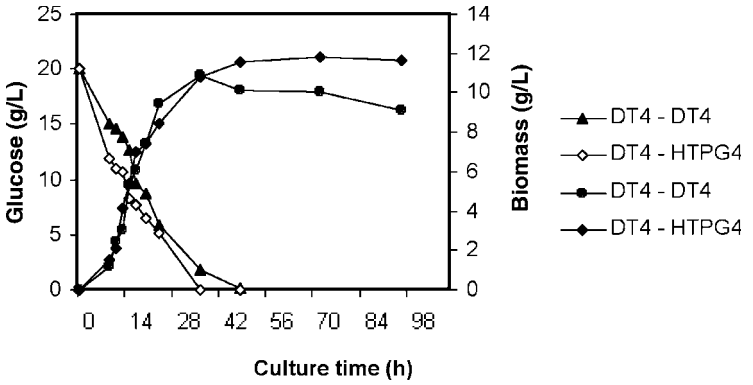

Fig. 5. Evolution of biomass production and glucose consumption (g/L) during batch culture of *P. canescens* 10-10c in a 5-L bioreactor fitted with either DT4-DT4 or DT4-HTPG4. The culture medium contained 2% glucose, 1% peptone, and 1% yeast extract. The peripheral speed was 1.82 m/s, and the aeration rate was 1 vvm.

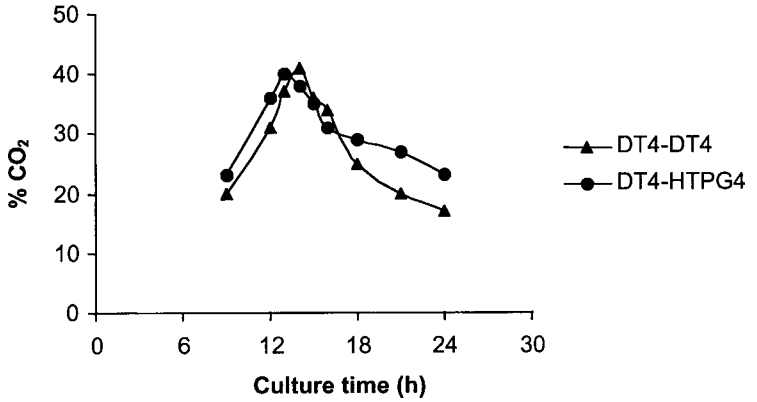

Fig. 6. CO_2 production during growth of *P. canescens* 10-10c in a 5-L bioreactor. The aeration rate was 1 vvm, the peripheral speed was 1.82 m/s, and the bioreactor was fitted with DT4-DT4 or DT4-HTPG4 impeller combinations.

Effect of Impeller Combination on Xylanase Production

The cultivations of *P. canescens* were then carried out in culture medium selected for xylanase production, in the presence of the two different combinations of impellers and at different aeration rates and agitation speeds. The presence of insoluble substrate (wheat bran) did not allow us to directly follow the biomass evolution. The production of CO_2 was used as an indirect indicator of growth. Figure 6 gives the results obtained for an aeration rate of 1 vvm and an agitation speed of 600 rpm. In both cases, CO_2 concentration in gas exhaust climaxed at about 12–14 h of culture. The maximum concentration reached was similar for the two impeller combinations. After 18 h of cultivation, the level of CO_2 produced appeared significantly lower for the DT4-DT4 combination. This

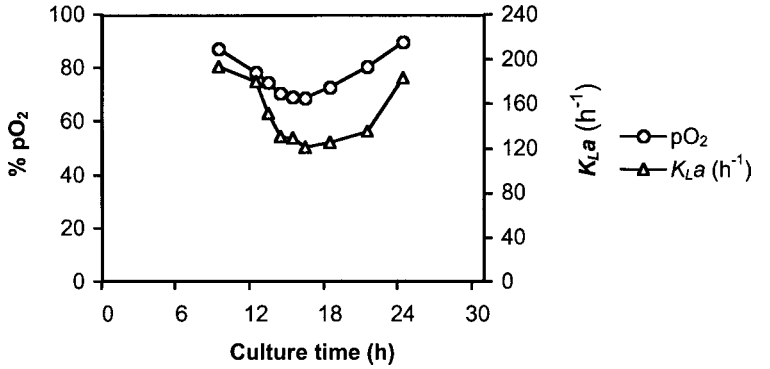

Fig. 7. Evolution of pO_2 and K_La (h^{-1}) during growth of *P. canescens* 10-10c in a 5-L bioreactor. The aeration rate was 1 vvm, the peripheral speed was 1.82 m/s, and the bioreactor was fitted with DT4-DT4 impeller combination.

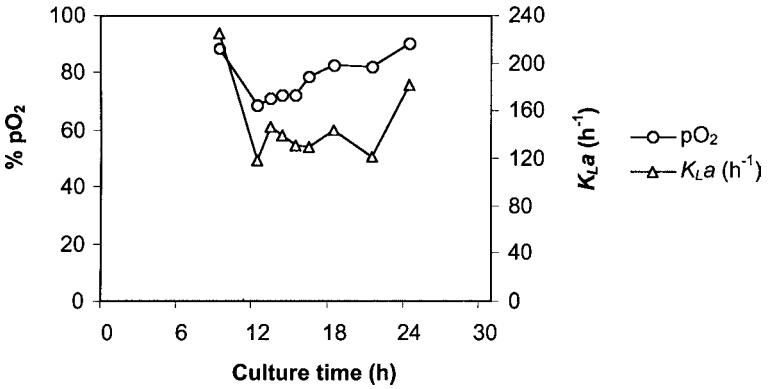

Fig. 8. Evolution of pO_2 and K_La (h^{-1}) during growth of *P. canescens* 10-10c in a 5-L bioreactor. The aeration rate was 1 vvm, the peripheral speed was 1.82 m/s, and the bioreactor was fitted with a DT4-HTPG4 impeller combination.

result confirms the presence of a less active biomass possibly resulting from a higher shear stress.

The pO_2 and K_La evolutions during cultivation in the first 24 h of culture are shown in Figs. 7 and 8. The pO_2 remained for both impeller combinations over 50% of saturation during growth. The U-shaped curve for K_La was already described in preceding work *(14,15)* and attributed to modifications of broth viscosity. The viscosity developed by substrate particles and biomass progressively increased during the growth phase. Therefore, the K_La values were decreased from 225 h^{-1} for the DT4-HTPG4 combination and 193 h^{-1} for the DT4-DT4 at 9 h of culture to 134 and 128 h^{-1}, respectively, between 12 and 20 h of culture. In a second phase, the substrate particles were progressively hydrolyzed, leading to lower viscosity and better oxygen transfer *(14)*.

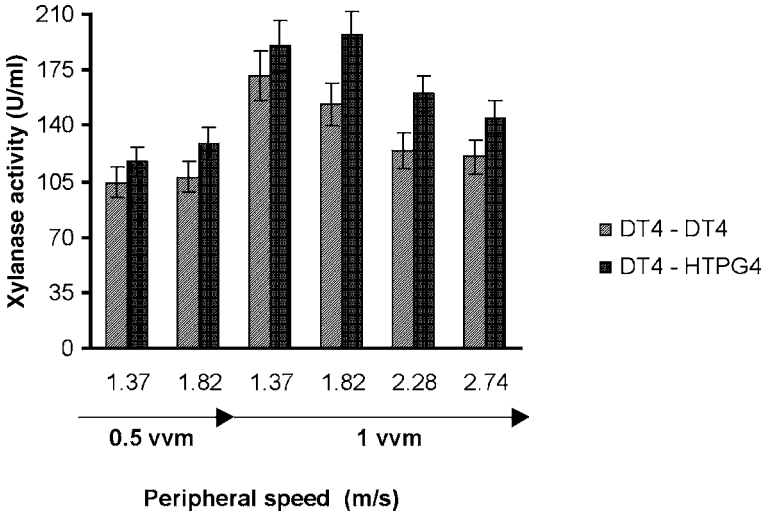

Fig. 9. Evolution of xylanase production (U/mL) after 120 h of culture of *P. canescens* 10-10c in a 5-L bioreactor fitted with either DT4-DT4 or DT4-HTPG4, at different peripheral speeds. The aeration rate was 0.5 and 1 vvm.

Xylanase production was analyzed in the different culture conditions. Xylanase production obtained after 120 h of culture at the different agitation speeds for an aeration rate of 0.5 or 1 vvm is shown in Fig. 9. At all the agitation speeds tested, xylanase production was higher for the DT4-HTPG4 combination than the DT4-DT4 combination. The best enzyme production was observed at 600 rpm. In these conditions, the combination DT4-HTPG4 gave an enzyme activity (198 U/mL), 23% higher than the DT4-DT4 combination (154 U/mL). Figure 9 also shows that improving aeration rate at a peripheral speed of 1.37 or 1.82 m/s has a positive effect on xylanase production. However, with the DT4-DT4 combination, at an agitation speed higher than 1.37 m/s and 1 vvm airflow rate, enzyme production decreased. The same phenomenon was observed for the other combinations but only for a peripheral speed of 1.82 m/s. The time course of xylanase production under these conditions and at 2.28 m/s is shown in Figs. 10 and 11. These data showed that the main differences between the production kinetics of the enzyme in the two combinations occurred between 24 and 48 h of culture.

Discussion

The influence of the two combinations of impellers on K_La, (two DT4 impellers and one DT4 impeller below and one HTPG4 above) was first tested in a culture medium used for xylanase production by *P. canescens* 10-10c. At all agitation speeds and aeration rates, the DT4-HTPG4 combination gave the best K_La values. The positive effect of this combination on K_La was more effective at low peripheral speed. The pumping action cre-

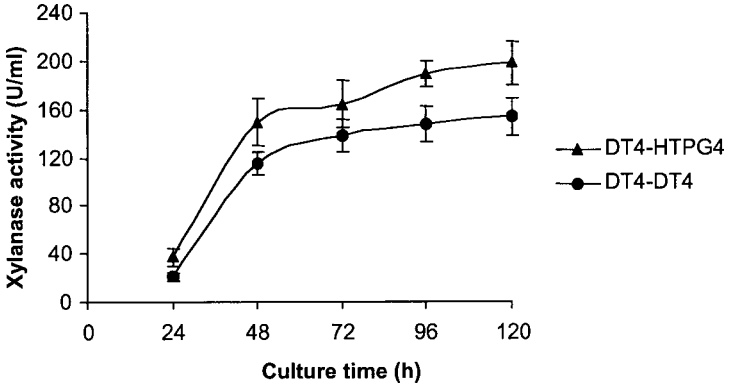

Fig. 10. Evolution of xylanase production (U/mL) during batch culture of *P. canescens* 10-10c in a 5-L bioreactor fitted with either DT4-DT4 or DT4-HTPG4. The peripheral speed was 1.82 m/s, and the aeration rate was 1 vvm.

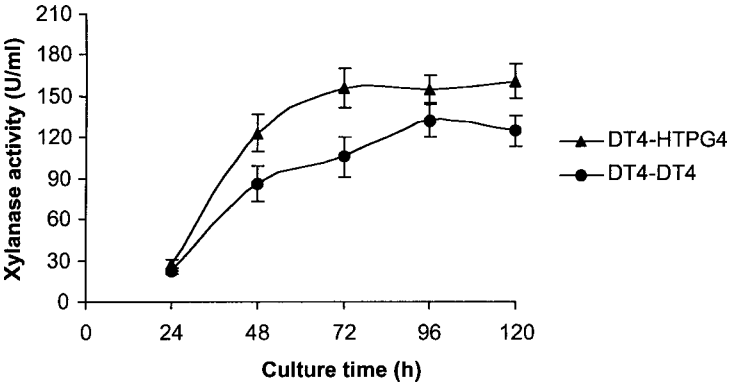

Fig. 11. Evolution of xylanase production (U/mL) during batch culture of *P. canescens* 10-10c in a 5-L bioreactor fitted with either DT4-DT4 or DT4-HTPG4. The peripheral speed was 2.28 m/s, and the aeration rate was 1 vvm.

ated by HTPG4 in the high position was more effective than the shear forces created by DT4. At high agitation speed, the difference between the two combinations was minimal owing to the increasing effect of the shear forces. Nevertheless, the effect of pumping for HTPG4, inducing higher circulation of bubbles and gas holdup in the combination of DT4-HTPG4, demonstrated better oxygen transfer than DT4-DT4. Our results agree with other studies reported in the literature, in which DT combined with the axial impeller above was recommended. Myers et al. *(20)* demonstrated that the use of multiple radial-flow impellers in gas-liquid reactors leads to poor top-to-bottom mixing because of zoning that occurs between impellers. Zoning is eliminated when high-efficiency impellers (HE-3) are used above the dispersing impeller, and these impeller systems reduce gassed blend times to less than half the value of multiple

radial-flow impeller systems. Arjunwadkar et al. *(21,22)* showed that among nine impeller combinations studied, the combination DT-PTD in which DT is mounted below and PTD is mounted above, was the optimum combination for effective gas dispersion, higher gas holdup, and maximum mass transfer coefficient at comparatively lower power input.

These results on $K_L a$ performance were exploited on xylanase production by *P. canescens* 10-10c cultivation. Previous studies performed in our laboratory demonstrated the sensitivity of *P. canescens* 10-10c to hydrodynamic stress. Biomass after 24 h of culture decreased by almost half at 1200 rpm compared with 450 rpm (3.75 and 7.0 g/L, respectively) *(15)*. The objective was to test the influence of the impeller combinations on xylanase production, biomass, pO_2, and $K_L a$ evolutions during cultivation of *P. canescens* in the presence of the two different combinations of impeller.

The use of the axial impeller improved xylanase production compared with the radial impeller. This effect was mainly owing to the lower shear forces generated by the HTPG4 impeller. Increased agitation speeds led to increased shear forces, which disrupted fragile microbial tissue, as shown by biomass and CO_2 production evolutions, and had a marked influence on xylanase production *(23)*. These results confirmed those obtained by Gaspar et al. *(14,15)*, who found that the PBT8 impeller had relatively greater xylanase yield (844 U/mL) than the disk-mounted blade turbine (722 U/mL) after 144 h of culture time; however, pO_2 evolution was similar in both cases.

The harmful effect of the shear forces on growth and enzyme production was demonstrated for xylanases and cellulases in the literature with different fungi *(11,13,24,25)*. This damage probably occurs in the regions of high shear rates, near the tips of the impeller blades. Wase et al. *(11)* has suggested that these high shear rates provoke mycelia break which first prevents the production of the enzymes, and second, leads to the probable simultaneous liberation of an extracellular enzyme inhibitor. Many researchers have reported a similar influence of shear forces and hydrodynamic stress on filamentous microorganisms *(26,27)*.

Conclusion

The effects of impeller configuration, aeration rate, and agitation speed on $K_L a$ and xylanase production were studied in dual-impeller systems with the new impeller design HTPG4 in the upper position and a DT4 turbine in the lower position. The oxygen transfer rates and xylanase production obtained with this combination were compared with those obtained with two Rushton turbines DT4 in similar conditions in *P. canescens* culture. $K_L a$ values increased with increasing agitation speed and aeration rates, and $K_L a$ values for the DT4-HTPG4 combination were better than those obtained for DT4-DT4, especially at low agitation speed. In addition, at high agitation speed, DT4-HTPG4 showed better performance for shear-sensitive biologic systems as demonstrated in *P. canescens*

cultivation in which DT4-HTPG4 produced 23% higher enzyme than DT4-DT4 at 600 rpm and 1 vvm. In general, the choice of geometry of HTPG4 impeller, which had a lower shear stress and efficient oxygen transfer, may be more suitable for microorganism culture processing, for which the issue of shear stress is especially critical.

Acknowledgments

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