Polygalacturonase Production by *Aspergillus sp.* in Air-Lift and Agitated Fermentor

Heydarian, Seyed Mohammad*+

Department of Biotechnology, Iranian Research Organisation for Science and Technology (IROST), P.O. Box 15815-3535 Tehran, I.R. IRAN

Bonakdarpour, Babak; Mirhoseini, Jalal

Deaprtment of Chemical Engineering, Amirkabir University of Technology, Tehran, I.R. IRAN

ABSTRACT: *Polygalacturonase production under different shear stress environments by Aspergillus sp. was studied. It was found that the rate of enzyme production in the stirred tank fermentor decreased with increasing the stirrer speed in the range 300-750 rpm. On the other hand, in a split cylinder air-lift fermentor, the rate of polygalacturonase production slightly increased with increasing in air rate from 1.5 to 2 v.v.m (volume air/culture volume/minute). The maximum enzyme titres at the end of the cultivation period in the airlift fermentor were 35 unit/ml and at least twice of that obtained in agitated fermentors. Measurement of main hyphal length in agitated and air-lift fermentors suggested break up of the mycelia in the higher shear environment of stirred fermentor especially at the higher agitation rates. The similarity in the trend of the enzyme production and main hyphal length suggested a possible relationship between the morphology and productivity of the Aspergillus sp. used in the present study.*

KEY WORDS: *Air-lift fermentor, Filamentous microorganisms, Morphology, Polygalacturonase, Shear.*

INTRODUCTION

Air-lift fermentor exhibit lower rates of oxygen mass transfer and mixing compared to agitated bioreactors and therefore their use for industrial production of enzymes could result in oxygen deficiency of the fermentation broth and inadequate bulk mixing. For these reasons stirred tank bioreactors are usually the preferred choice for industrial production of enzymes. On the other hand, compared to stirred bioreactors, air-lift fermentors have a simpler design, have a lower capital and operating costs and exhibit a lower shear environment. For the latter reason, air-lift fermentors have been used successfully in fermentation of shear sensitive microorganism in high viscosity non-Newtonian fermentation broths [1,2].

There are few reports about effect of agitation on morphology and polygalacturonase production of Aspergillus spices. *Sonia et al.* [3] reported higher

* *To whom correspondence should be addressed.*
+ *E-mail: heydarian@irost.org*
1021-9986/09/1/103 5/$/2.50

polygalacturonase production for looser morphology of *A. niger* pellets favoring the nutrient transfer. *Fredrich et al.* [4] reported that agitation speed did not change the morphology of *A. niger* mutant although it had a marked influence on polygalacturonase production. *Tari et al.* [5] studied effect of agitation on morphology and polygalacturonase production of *A. sojae* pellet and observed that small pellets resulted in higher enzyme activities under the condition of low agitation speed.

The aim of this study has been to assess the effect of shear on polygalacturonase production using a strain of *Aspergillus* (ATHUM-3482) by studying the production of this enzyme in stirred and air lift fermentors

MATERIAL AND METHOD

Microorganism

The organism used in this study, *Aspergillus sp.*, ATHUM-3482, was kindly denoted by Galiotou-Pnayotou (Agricultural university of Athens).

Culture condition:

Medium contained per liter: 11 g Di-ammonium hydrogen phosphate, 1 g magnesium sulfate, 1 g Calcium chloride, 1 g Di-Sodium Hydrogen Phosphate and 50 g sugar beet pulp screened by mesh 30. Silicone oil was added to the medium as antifoam in all the agitated and airlift fermentor and some of the shake flask experiments.

For shake flask experiments, a 250 ml flask containing medium was incubated with spore suspension at 30 °C and 180 rpm on an Orbital shaker (Clim-O-shake, Kuhner). The working volume of flask was 40 mL.

For agitated fermentor experiments, a 20 liter stirred fermentor (CHEMAP) was used with working volume of 13 liter. The ratio of impeller diameter to the tank diameter was 0.35. Agitation was provided by a set of two Rushton turbines mounted on a central shaft. Experiments were carried out at 4 different agitated speeds namely 300, 350, 500 and 750 rpm. The aeration rates were 1 v.v.m (volume air/culture volume/minute) throughout of the fermentation for all experiments.

For air-lift fermentor experiments, a split cylinder air-lift constructed of pyrex glass with 6.5 liter working volume and 10 mm internal diameter and consisting of a riser tube of 0.6 mm internal diameter was used. Experiments were carried out at aeration rates of 1.5 and 2 v.v.m.

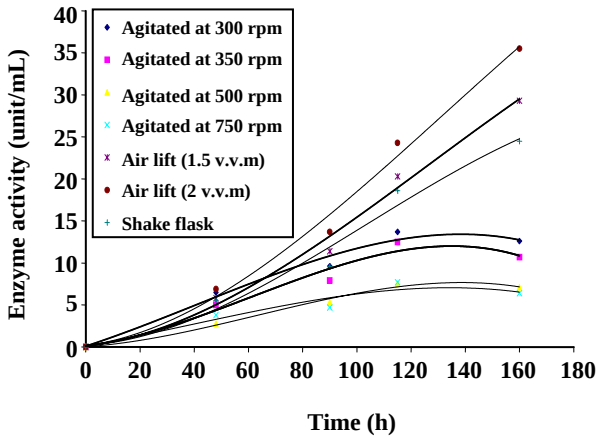

Fig. 1: Comparison of polygalacturonase production between agitated and air-lift fermentors.

Enzyme assay

Polygalacturonase activity was measured according to the procedure given by *Maldona* and *Strasser de Saad* [6], and the concentration of reducing sugars was determined by the dinitro-salicilic acid (DNS) method [7].

Main Length Measurement

A Neubauer Improved Bright-line slide (Tief Depth profondeur, 0.1 mm) was used for measurements of main length of the mycelium. Neubauer slide was filled with samples and main length of the mycelium was measured with microscope.

RESULTS AND DISCUSSION

Comparison of Enzyme production between shake flask and agitated fermentor at different stirrer speeds

Time course of fermentation for average enzyme production at different stirrer speeds is shown in Fig. 1. And the average of several shake flask experiments are also presented for comparative purposes. Results of the shake flask runs show increase in the concentration of polygalacturonase enzyme production up to 25 unit/mL after 160 hours of fermentation. On the other hand the enzyme production in stirred bioreactors peaked at 140 hours, with the highest enzyme activity being less than 14 unit/mL at stirrer speed of 300 rpm. It was thought that part of the reason for the lower enzyme titres obtained in the stirred fermentor can be the presence of antifoam in the medium used in agitated fermentor experiments. In order to shed light on this matter shake flask experiments were repeated with media containing antifoam.

Table 1: Polygalacturonase activity (unit/mL) for shake flask experiments for different antifoam concentration.

Fermantation time (h)	Without antifoam	Antifoam (2 mL/L)	Antifoam (3mL/L)	Antifoam (4mL/L)	Antifoam (5mL/L)
72	9.1	9.5	8.3	8.3	7.4
96	13.2	13.2	11.9	14.0	13.3
120	18.5	18.1	17.4	17.8	19.0

Results presented in table 1 showed that antifoam was not the reason for lower enzyme production obtained in the agitated fementor.

Tripathi et al. [8] also reported higher production of calcium gluconate by *Aspergillus niger* in shake flask compared to bioreactor. On the other hand, *Arif et al*. [9] found lower production of kogic acid with Aspergillus fermentation in shake flask compared to agitated fermentor and suggested that the lower oxygen transfer in shake flask compared to stirred bioreactor is responsible for lower productivity in the former. The *Aspergillus sp* used in our study seems to be shear sensitive and therefore lower enzyme production was obtained in agitated fermentor. While in most of fungal fermentation, a high agitation rate is necessary to provide adequate oxygen transfer [10-12], mechanical forces can cause mycelia damages [13,14]. Thus the agitation rate is limited to a range to avoid undesired effects. This limitation is strain specific and it is not yet clear whether the cell physiology or the change of fugal morphology is the reason for variation in productivity [15].

Comparison of enzyme production at different stirrer speeds in the agitated fermentor also confirmed shear sensitivity of this fugal species (Fig. 1). Decreasing the strirrer speed from 750 to 300 rpm has resulted in increase in enzyme titre from around 7 unit/mL to a bit less than 14 unit/mL. Increasing stirrer speed leads to higher shear rates which on the one hand leads to better rates of oxygen transfer and mixing, whereas on the other hand it can lead to damage to the mycelial structure of the fungi. The latter phenomenon seems to have governing rule in the present work. This phenomenon has also been observed in some other work with filamentous organisms [16, 17].

Comparison of Enzyme productivity between air- lift and agitated fermentor

Polygalacturonase production using the *Aspergilus sp.* was also carried out at aeration rates of 1.5 and 2 v.v.m in the air-lift fermentor. The results, presented in Fig. 1, show that the enzyme production in air-lift fermentor was slightly higher at the higher aeration rate employed, which is probably the result of higher oxygen transfer rates at the higher air rate. Comparison of the enzyme titres obtained in the air-lift experiments with the highest enzyme titre obtained in the stirred fermentor at stirrer speed of 300 rpm shows nearly twice the rate of polygalacturonase production in the air-lift Fermentor. Similar to the trend of enzyme production obtained in the shake flask, no peak is observed in the enzyme production with time graph at both air rates employed in the air-lift fermentor. This result is in agreement with lower itaconic acid production by *Aspergillus terreus* [18] and cellulose production by *Aspergillus fumigatus* [19] in agitated fermentors compared to air-lift fermentor. In previous reports morphology has not been examined to show the possibility of mycelia damage for lower productivity in agitated vessels compared to air-lift fementors. We examined the morphology in both agitated and air-lift fermentors and showed that damage due to shear could be the reason for this variation.

Comparison of fungal morophology in air-lift and agitated fermentors

In order to further investigate the effect of shear on the morphology of the *Aspergilus sp.* used in the present study for polygalacturonase production, the main length of hyphae, as one characteristics of fungal morphology was determined in the agitated and air-lift bioreactor under different operating conditions. The main length of the *Aspergilus sp.* was measured in the agitated fermentor at 500 and 750 rpm and in the air-lift fermentor at 1.5 v.v.m All experiments carried out in duplicate and reproducibility of results was good and mean standard error was less than 10 %. The results presented in Fig. 2 shows that at both stirrer speeds in the stirred tank fermentators the main length of the hyphae increased till 120 hr thereafter slightly decreased. The decrease in the length being more pronounced at the higher agitation rate.

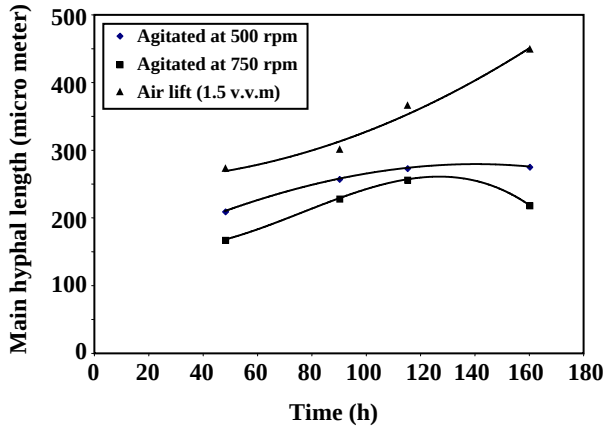

Fig. 2: Changes of Main Hyphal Length of mycelia during fermentation for agitated fermentor at 500 and 750 rpm and air-lift fermentor aerated at 1.5 v.v.m.

Also slightly higher main length was obtained at stirrer speed of 500 rpm compared to 750 rpm. On the other hand in the air-lift bioreactor experiments the increase in the main length continued until 160 hr. The main length of the mycelia was also higher in air-lift fermentor compared with stirred tank fermentor throughout of cultivation period for both stirrer speeds. These results are in agreement with the trend of polygalacturonase production obtained under these conditions and suggest higher shear damage to mycelia with agitated compared to air-lift fermentor and a possible relationship between the morphology and productivity of the *Aspergilus sp.* used in the present study.

CONCLUSIONS

polygalacturonase production by *Aspergilus sp.* in air-lift bioreactor was more than twice of agitated bioreactors. This higher production of Polygalacturonase was related with higher hyphal main length in the air-lift bioreactor. Higher stirrer speeds in agitated fermentor also resulted in both lower polygalacturonase production and hyphal main length. These results suggest that for polygalacturonase production by *Aspergilus sp.* using air-lift fermentor is a potentially better bioreactor by providing a more shear-friendly environment.

Acknowledgements

Financial support of Iranian Research Organization for Science and Technology (IROST) and assistance of personnel of mini-pilot lab including Mr. F. Hormozi, Mr. A. Sheikhinejad and Mr. H. Moradi in Biotechnology department of IROST gratefully acknowledged.

Received :6th August 2007 ; Accepted : 5th August 2008

REFERENCES

[1] Rodriguez, A., Paterson, R.R.M., Lima, N. and Texeria, J.A., Laccase Activity from the Fungus *Trametes hirsuta* Using an Air-Lift Bioreactor., *Lett. Appl. Microbiol.*, **42**, 612 (2006).

[2] Rocha Valadez, J.A., Albiter, V., Caro, M.A., Serrano Carreon, L. and Galindo, E., A Fermentation System Designed to Independently Evaluate Mixing and/or Oxygen Tension Effects in Microbial Processes: Development, Application and Performance, *Bioprocess Biosyst. Eng.*, **30**, 115 (2007).

[3] Sonia, C., Gustavo, P., Lilian, S. and Hebe Labarthe, H., Influence of Metal Ions on Pellet Morphology and Polygalacturonase Synthesis by *Aspergillus niger* 3T5B8, *Braz. J. Microbiol.*, **34**, 16 (2003).

[4] Fredrich, J., Cimerman, A. and Steiner, W., Submerged Production of Pectolytic Enzymes by *Aspergillus niger*: Effect of Different Aeration/ Agitation Regimes, *Appl. Microbiol. Biotechnol.*, **31**, 490 (1989).

[5] Tari, C., Gogus, N. and Tokalti, F., Optimization of Biomass, Pellet Size and Polygalacturonase Production by *Aspergillus sojae* ATCC 20235 Using Response Surface Methodology, *Enzyme. Microbial. Tech.*, **40**, 1108 (2007).

[6] Maldonado, M.C. and Strasser, S., Production of Pectinesterase and Polygalacturonase by *Aspergillus niger* in Submerged and Solid State Systems, *J. Ind. Microbiol. Biotechnol.*, **20**, 34 (1998).

[7] Miller, G.L., Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugars, *Anal. Chem.*, **31**, 426 (1959).

[8] Tripathi, C.K., Rastogi, S., Bihari, V. and Basu, S.K., Production of Calcium Gluconate by Fermentation, *Indian. J. Exp. Biol.*, **37**, 1 (1999).

[9] Ariff, A.B., Rosfarisan, M., Herng, L.S., Madihah, S. and Karim, M.I.A., Kinetics and Modeling of Kojic Acid Production by *A. flvus* in Batch Fermentation, *World J. Microbiol. Biotechnol*., **13** (1997).

[10] Heydarian, S.M., Lilly, M.D., Ison, A.P. and Ayazi Shamlou, P.A., Turbulent Breakage of Filamentous Bacteria in Mechanically Agitated Batch Culture, *Chem. Eng. Sci.*, **55**, 1775 (2000).

[11] Cui, Y.Q., Okkerse, W.J., Van Der Lans, R.G. and Luyben, K .C., Modeling and Measurements of

Fungal Growth and Morphology in Submerged Fermentations, *Biotechnol. Bioeng.*, **60**, 216 (1998).

[12] Heydarian, S.M., Lilly, M.D. and Ison, A.P., The Effect of Culture Conditions on the Production of Erythromycin by *Saccharopolyspora Erythraea* in Batch Culture, *Biotech. Lett.*, **18**, 1181(1996).

[13] Ayazi Shamlou, A. P., Makagiansar, H. Y., Ison, A.P., Lilly, M.D. and Thomas, C.R., Breakage of Filamentous Microorganisms in Submerged Culture in Mechanically Stirred Fermentor, *Chem. Eng. Sci.*, **49**, 2621 (1994).

[14] Gibbs, P.A., Seviour, R.J. and Schmid, F., Growth of Filamentous Fungi in Submerged Culture: Problems and Possible Solutions, *Critical Review in Biotechnology*, **20**, 17 (2000).

[15] Wang, L., Ridgway, D., Gu, T. and Moo Young, M., Bioprocessing Strategies to Improve Heterologous Potein Production in Filamentous Fungal Fermentations, *Biotechnol. Adv.*, **23**, 115 (2005).

[16] Li, Z.J., Shukla, V., Wenger, K.S., Fordyce, A.P., Pedersen, A.G. and Merten, M.R, Effects of Increased Impeller Power in a Production Scale *Aspergillus Orazae* Fermentation, *Biotechnol. Prog.*, **18**, 437 (2002).

[17] Oncu, S., Tari, C. and Unluturk, S. Effect of Various Parameters on Morphology, Rheology and Polygalacturonase Production by *Aspergillus sojae* in a Batch Bioreactor, *Biotechnol. Prog.*, **23**, 836 (2007).

[18] Yahiro, K., Takahamara, T., Jal, S., Park, Y. and Okabe, M., Comparison of Air-Lift and Stirred Tank Reactors for Itaconic Acid Production by *Aspergillus terreus, Biotechnol. Lett.*, **19**, 619 (1997).

[19] Wase, D.A.J., McManamey, W.J., Raymahasay, S. and Valid, A.K., Comparisons between Cellulose Production by *Aspergillus fumigatus* in Agitated Vessels and in an Air-Lift Lermentor, *Biotechnol. Bioeng.*, **27**, 1166 (1985).