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Scanning Electron Microscopic Analysis of *Aspergillus niger* Pellets and Biofilms under Various Process Conditions

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Abstract: *Aspergillus* spp are among the powerful microbes involved in environmental management. The growth of *Aspergillus niger* varies in the form of pellets to biofilm. Pelleted form of growth has been observed in the media without any support, whereas growth nature has been shifted (pelleted to biofilm form) by incorporating solid support in the medium. Scanning electron microscopy (SEM) has proved to be a precious and valuable implement for morphological as well as structural characterization of fungal pellets and biofilms. It has been observed that polyester sheet act as a better solid support for development of thick biofilm having lesser interstitial voids and more structured channel which provides conditions for improved mass transfer needed for effective and efficient application of *Aspergillus* system in industrial and environmental management process.

Key words: Muslin cloth · Polyester sheet · Mass transfer · SEM · *Aspergillus niger* · Fungal growth

INTRODUCTION

Aspergillus spp are the major agents of decomposition and decay of lignocellulosic wastes and thus possess the capability to produce a broad range of enzymes. High levels of β-glucosidase are important for the complete conversion of lignocelluosic biomass. *Aspergillus* spp are also known to be good and efficient bioremediation agent. Morphology of the fungal system has a significant influence on the mass transfer and turn over processes in submerged cultures [1], therefore it is necessary to analyze the structural and morphological form of environmentally suitable microbes. *A niger* is better known in the form of pelletized growth under submerged cultivation. Pellets are highly entangled dense masses of hyphae. They consist of an outer shell of growing hyphae and an inner mass of non growing mycelium [2, 3]. Biofilm terms are generally applied for bacterial growth, but in few fungal cases too, it has been recently reported. Biofilm formation consists of spore adhesion, which depends on both its rough surface and adhesive substrates that form a pad between spore and support [4]. But, recent literature supported growth of *A. niger* in the biofilm form under solid support. It has also been reported that biofilm fermentation produces higher enzyme yields than SmF with lower biomass yields [5].

The present paper evaluated and compared the growth of *Aspergillus niger* on polyester sheet and muslin cloth like solid support and without support in lactose based media through scanning electron microscopy.

MATERIALS AND METHODS

All the chemicals and reagents were purchased from Himedia, India. *Aspergillus niger* NCIM777 strain was procured from National Chemical Laboratory (NCL), Pune. Fungal spores from a stock, kept at 4°C in 20% (v/v) glycerol. *Aspergillus* cultures were grown on PDA slants at 28°C for 4 days. Slants were maintained at 4°C and subcultured at monthly intervals. Batch experiments were performed in 250 ml Erlenmeyer flasks containing 100 ml of production media containing (g/l) Urea,0.3; $(NH_4)SO_4$,1.4; KH_2PO_4,2.0; $CaCl_2.2H_2O$,0.4; MgSO4.$7H_2O$,0.3; Peptone,1.0; Tween-80,0.2; $FeSO_4.7H_2O$,0.005; $MnSO_4.7H_2O$, 0.0016; $ZnSO_4.7H_2O$,0.0014; $CoCl_2.6H_2O$,0.02 with lactose 10, as

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carbon source at initial pH 5.0. For biofilm study, washed, sterilized and round shaped muslin cloth with 3 hole of 0.5cm in diameter has been incorporated in the lactose based media while in other set of experiment, washed, autoclaved rectangular sheet of polyester has been incorporated in the lactose based production medium. A separate set of experiment has also been performed using lactose based production media without any solid support. All the experimental flasks have been autoclaved and inoculated with ~ 4.0 ml of freshly prepared PDB (Potato dextrose broth) culture solution (0.56g/l cell dry weight) of *Aspergillus niger*. All experiments were conducted on incubator shaker at 30°C with 180 rpm. The morphological and structural features of *Aspergillus niger* biofilm were analyzed through scanning electron microscope model LEO-435 VP.

RESULTS AND DISCUSSION

From the above experimental studies, two types of growth morphology have been observed, pelleted (without any support containing medium) and biofilm (medium with solid support) form. For the morphological and structural studies, scanning electron micrography has been performed. Microbial biofilms and fungal pellets have been studied with increasing curiosity during the past few years. Although the huge majority of published papers refer only to bacterial or yeast-like biofilms, filamentous fungi could be included as biofilm forming microorganisms, since they are naturally adapted. Scanning electron microscopy has proved to be a precious and invaluable tool for analyzing the structure and growth of fungal pellets and biofilms. Fungal pellets are a core of densely packed hyphae, surrounded to a greater or lesser exent by a more annular dispersed or hairy regions containing radially growing hyphae, which can vary in size between several hundred micrometers to several millimeters, whereas typical microbial biofilm structure includes a complex three dimensional structural design characterized by interstitial voids and water channels with cells usually encapsulated within an extracellular matrix [6].

In a nut shell biofilm mycelia showed an orderly distribution forming surfaces and inner channels, while pellets showed highly intermined superficial hyphae and a densely packed deep mycelium [5]. Pellets had vague holes which represented the lesser portion of the total area, however because they are not factual channels, compacting and stressed hyphae were manifest around them as evident from Figs. 1, 2. It has also been shown that large number of spores are scattered around major section. However, in culture growth systems, *Aspergillus* pellets consist of an outer shell of growing hyphae and an inner mass of non growing mycelium. The thickness of the outer growing layer of pellets is considered to be limited by nutrient diffusion rate [7]. Researchers have suggested that the formation of pellets originated from the adherence of germinated spores to solid particles in medium. The attached solid particles were also digested during the fungal fermentation and resulted in the formation of the smooth and hollow pellets [8].

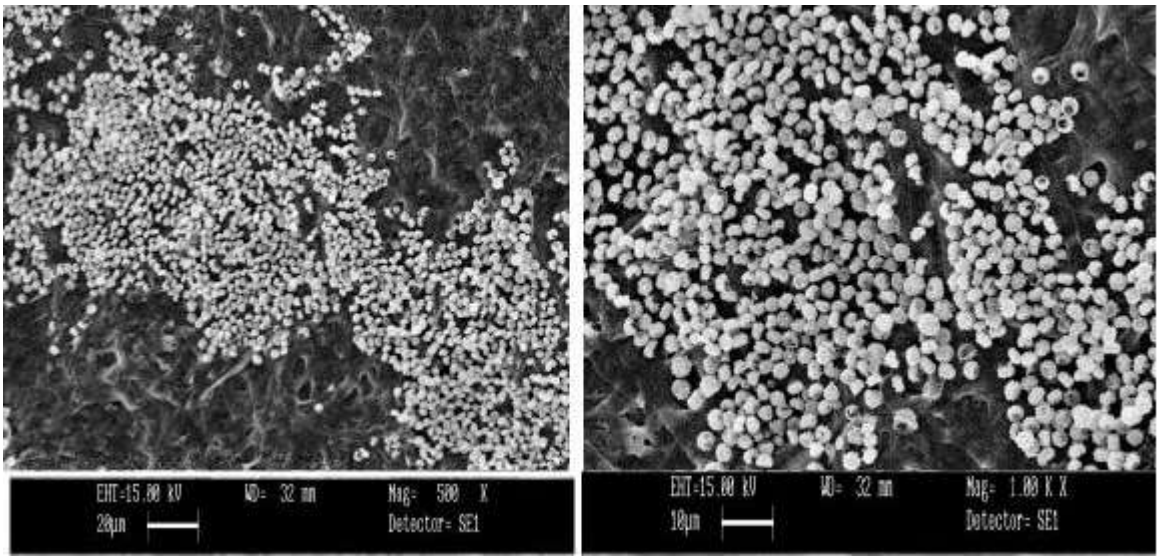

Fig. 1, 2: Scanning electron micrograph of *Aspergillus niger* pellets at 500X and 1000 X magnification respectively

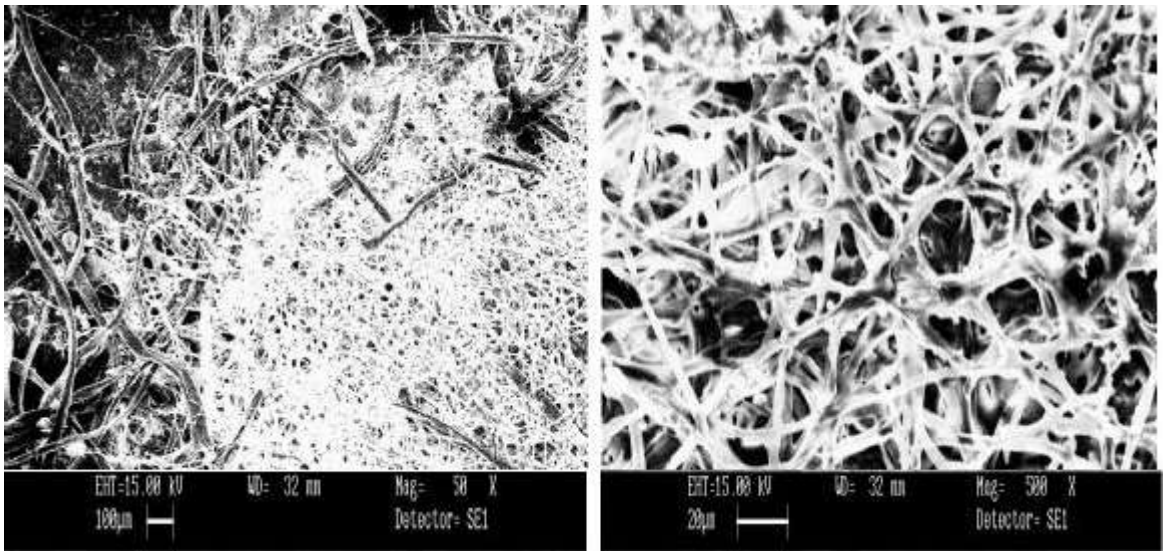

Fig. 3,4: Scanning electron micrograph of *A. niger* biofilm on muslin cloth solid support at 50 X and 500X magnification respectively

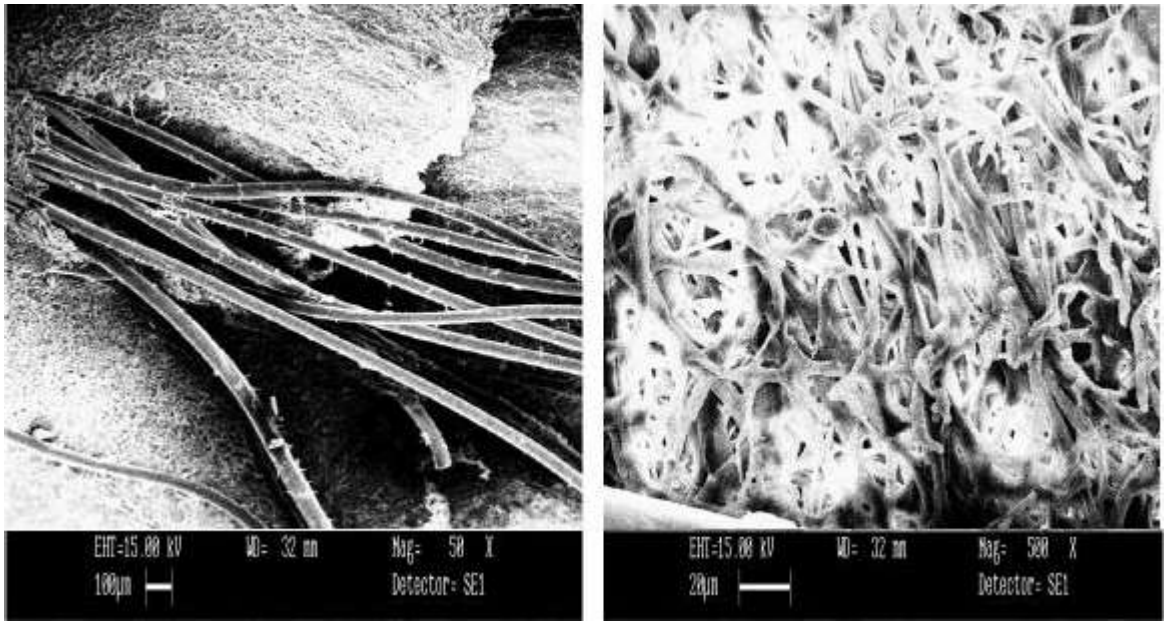

Fig. 5,6: Scanning electron micrograph of *A. niger* biofilm on polyester sheet solid support at 50X and 500X magnification respectively

Biofilms revealed surface heterogeneity and interactions voids as well as also exhibited well structured channels with swollen hyphae around as explained in Figs. 3-6. When compared the biofilms developed on solid support and pellets of *A. niger* we clearly observed a different spatial growth harmonization when fungus adhere to the surface. This synchronization is the result of steric interactions between hypha and tips, when in contact with the surface. At tiny distances, interactions between tip and hyphae involve a local spatial rearrangement, resulting in reduction of the tip elongation rate. As a result, a control in maximum biomass surface density has observed [9].

Upon comparing the scanning electron micrograph of *A. niger* biofilm on muslin cloth and polyester solid sheet support, huge amount of growth has been observed with polyester solid sheet support having smaller and lesser number of interstitial voids incorporated with

dense and compact network of inner channels whereas biofilm developed on muslin cloth holding bigger and more number of interstitial voids integrated with less dense network of inner channels as confirmed by Figs. 5, 6 which might be due to hard and compact structure of polyester sheet required for better surface adhesion under agitating condition. A channeled structure has a noticeable benefit in that it allows fluids and nutrients to pass through easily which ultimately enhances mass transfer which also proves the robust and sound relationship between growth morphology and product formation [10]. Therefore, fungal biofilms are considered more efficient than suspended mycelium in many production systems [11, 12].

In conclusion, this paper corroborated the structural and morphological differences between biofilms and pellets as well as in between biofilms of *A. niger*. Biofilms developed on polyester sheet are massive in nature with lesser interstitial voids and more channeled structure which improved nutrient and mass transfer compared to pelleted or filamentous mycelial form of fungal growth and ultimately these conditions improved fungal growth and production system for better application in environmental management process.

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REFERENCES

1. Parcel, E.M.R., J.L.C. Lope, J.A.S. Perez, J.M.F. Sevila and R. Christi, 2005. Effects of pellet morphology on broth rheology in fermentations of *Aspergillus terreus.* Biochemical Engineering J., 26: 139-144.
2. Domingues, F.C., J.A. Queinoz, J.M.S. Cabral and L.P. Fonseca, 2000. The influence of culture conditions on mycelial structure and cellulase production by *Trichoderma reesei* RUT C 30. Enzyme Microbial Technol., 26: 394-401.
3. Karns, G., E. Dalchow, G. Klappacha and D. Meyer, 1995. Formation and release of β-glucosidase by *Aspergillus highzinet* 43746 in correlation to process operations. Acta Biotechnologia, 6: 355-359.
4. Villena, G.K and M. Guiterrez -Correa, 2008. Morphological patterns of *Aspergillus niger* biofilms and pellets related to lignocellulolytic enzyme productivities.Letters in Applied Microbiol., 45: 231-237.
5. Villena, G.K. and M. Guiterrez -Correa, 2007. Production of lignolytic enzymes by *Aspergillus niger* biofilms at variable water activites, Electronic J. Biotechnol., pp: 10.
6. Stoodley, P., K. Sauer, D.G. Davies and J.W. Costerton, 2002. Biofilms as a complex differentiated communities. Annual. Review of Microbiol., 56: 187-209.
7. http://links.JSTOR.org/sici, JSTOR, Proceedings of the Royal Society of London, Series B, Biological Sci., 166: 1-3.
8. Troung, Q.T., N. Miyata and K. Iwahori, 2004. Growth of *Aspergillus oryzae* during treatment of cassava starch processing waste water with high contents of suspended solids. J. Bioscience and Bioengineering, 97: 329-335.
9. Ferret, E., J.H. Siméon, P. Molin, H. Jorquera, G. Acuña and R. Giral, 1999. Macroscopic growth of filamentous fungi on solid substrate explained by a microscopic approach, Biotechnology and. Bioengineering, 65: 512-522.
10. Villena, G.K., T. Fujikawa, S. Tsuyumu and M. Gutiérrez-Correa, 2010. Structural analysis of biofilms and pellets of *Aspergillus niger* by confocal laser scanning microscopy and cryo scanning electron microscopy, Bioresource Technol., 101: 1920-1926.
11. Fiedurek, J., 2001. Production of gluconic acid by immobilized in pumice stones mycelium of *Aspergillus niger* using unconventional oxygenation of culture. Biotechnol. Letters, 23: 1789-1792.
12. Talabardon, M. and S.T. Yang, 2005. Production of GFP and glucoamylase by recombinant and effects of fermentation conditions on fungal morphology and protein secretion. Biotechnol. Progress, 21: 1389-1400.