







### 3.5 Optimization of inoculum size

Optimum spore density (number of spores per unit weight of substrate) is important for SSF process. The maximal enzyme activity (466 U/mL) was noted at the 6 mL inoculum size of co-culture (*T. viride* and *G. lucidum*) (Fig. 5). Lower inoculum sizes shortened the microbial lag phase stage, whereas inoculum size beyond the optimum value increased the moisture factor that caused lower levels of enzyme formation due to the overcrowding of fungal spores [3-5]. *Aspergillus niger* grown under solid state fermentation conditions gave maximum enzyme activity (216.2 IU/g) at an inoculum size of 10% using wheat straw as the substrate [16]. In an earlier study Omojasola and Jilani [14] reported the maximum cellulase activity with an inoculum size of 8%.

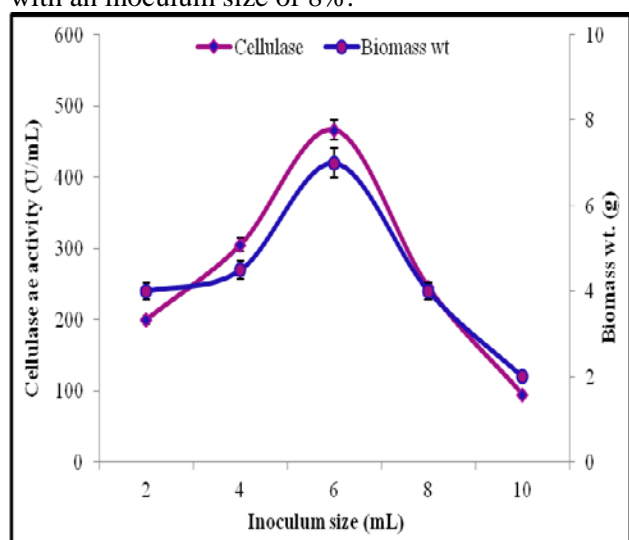


Fig.5. Cellulases activity on varying sizes of fungal inoculums

### 4 Conclusions

In conclusion attempt was made, to find the optimum fermentation conditions for successful cultivation of microbial co-cultures, and also towards an induced production of third most demanded industrially important enzyme cellulase. However, the suitability of the enzymes for biotechnological applications can be investigated through kinetic characterization of the purified enzymes as thermo-stability is a desired characteristic of an enzyme for its possible use in industry.

### Acknowledgments

The authors are grateful to the Department of Biochemistry and Molecular Biology, University of

Gujrat, Pakistan for providing financial support and laboratory facilities.

### References:

- [1] H.M.N. Iqbal, M. Asgher, I. Ahmed, and S. Hussain, "Media optimization for hyper-production of carboxymethyl cellulase using proximally analyzed agro-industrial residue with *Trichoderma harzianum* under SSF", *IJAVMS*, Vol. 25, 2010, pp. 37.
- [2] M. Irshad, Z. Anwar, and A. Afroz, "Characterization of Exo 1, 4- $\beta$  glucanase produced from *Trichoderma viridi* through solid-state bio-processing of orange peel waste", *Advances in Bioscience and Biotechnology*, Vol. 3, 2012, pp. 580-584.
- [3] H.M.N. Iqbal, I. Ahmed, M.A. Zia, and M. Irfan, "Purification and characterization of the kinetic parameters of cellulase produced from wheat straw by *Trichoderma viride* under SSF and its detergent compatibility", *Advances in Bioscience and Biotechnology*, Vol. 2, No. 3, 2011a, pp. 149-56.
- [4] H.M.N. Iqbal, M. Asgher, and H.N. Bhatti, "Optimization of physical and nutritional factors for synthesis of lignin degrading enzymes by a novel strain of *Trametes versicolor*", *BioResources*, Vol. 6, 2011b, pp. 1273-1278.
- [5] M. Irshad, Z. Anwar, H.I. But, A. Afroz, N. Ikram, and U. Rashid, "The industrial applicability of purified cellulase complex indigenously produced by *Trichoderma viride* through solid-state bio-processing of agro-industrial and municipal paper wastes", *BioResources*, Vol. 8, No. 1, 2013, pp. 145-157.
- [6] V. Verma, A. Verma and A. Kushwaha, "Isolation and production of cellulase enzyme from bacteria isolated from agricultural fields in district Hardoi, Uttar Pradesh, India", *Advances in Applied Science Research*, Vol. 3, 2012, pp. 171-174.
- [7] R.E. Quiroz-Castañeda, E. Balcázar-López, E. Dantán-González, A. Martínez, J. Folch-Mallol, and C.M. Anaya, "Characterization of cellulolytic activities of *Bjerkandera adusta* and *Pycnoporus sanguineus* on solid wheat straw medium", *Electronic Journal of Biotechnology*, Vol. 12, No. 4, 2009, pp. 5-6.
- [8] H.M.N. Iqbal, S. Kamal, I. Ahmed, and M.T. Naveed, "Enhanced bio-catalytic and tolerance properties of an indigenous cellulase through xerogel immobilization",

*Advances in Bioscience and Biotechnology*,  
Vol. 3, 2012, pp. 308-313.

- [9] H.M.N. Iqbal, G. Kyazze, and T. Keshavarz, "Advances in the valorization of lignocellulosic materials by biotechnology: An overview", *BioResources*, Vol. 8, 2013, pp. 3157-3176.
- [10] S.P. Gautam, P.S. Bundela, A.K. Pandey, M.K. Awasthi, S. Sarsaiya, "Optimization of the medium for the production of cellulase by the *Trichoderma viride* using submerged fermentation", *International Journal of Environmental Sciences*, Vol. 1, 2010, pp. 656-665.
- [11] G.M. Ishfaq, S. Ahmed, M.A. Malana, and A. Jamil, "Corn stover-enhanced cellulase production by *Aspergillus niger* NRRL 567", *African Journal of Biotechnology*, Vol. 10, No. 31, 2011, pp. 5878-5886.
- [12] S. Pushalkar, K. Rao, and K. Menon, "Production of  $\beta$ -glucosidase by *Aspergillus terreus*", *Current Microbiology*, Vol. 30, No. 5, 1995, pp. 255-258.
- [13] A.J. Sami, M. Awais, and A.R. Shakoori, "Preliminary studies on the production of endo-1, 4- $\beta$ -Dglucanases activity produced by *Enterobacter cloacae*", *African Journal of Biotechnology*, Vol. 7, No. 9, 2008, pp. 1318-1322.
- [14] P. Omojasola, and O. Jilani, "Cellulase production by *Trichoderma longi*, *Aspergillus niger* and *Saccharomyces cerevisiae* cultured on plantain peel", *Research Journal of Microbiology*, Vol. 4, No. 2, 2009, pp. 67-74.
- [15] H. Sun, X. Ge, Z. Hao, and M. Peng, "Cellulase production by *Trichoderma* sp. on apple pomace under solid state fermentation", *African Journal of Biotechnology*, Vol. 9, No. 2, 2010, pp. 163-166.
- [16] M. Fadel, "Production physiology of cellulases and  $\beta$ -glucosidase enzymes of *Aspergillus niger* grown under solid state fermentation conditions", *OnLine Journal of Biological Sciences*, Vol. 1, No. 5, 2000, pp. 401-411.