Electrospun polyacrylonitrile nanofibrous membranes for chitosanase immobilization and its application in selective production of chitooligosaccharides

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ABSTRACT
Polyacrylonitrile nanofibrous membranes (PANNFM) were prepared by electrospinning from 10 wt.% of PAN solution and its surface was modified by amidination reaction. A new chitosanase degrading enzyme from Aspergillus sp. was covalently immobilized on PANNFM. Immobilization efficiency of 80% was achieved by activating PANNFM surface for 30 min followed by 2 h treatment with enzyme solution. The optimum temperature and pH for immobilized enzyme were 50 °C and 5.8, respectively. The immobilized chitosanase retained >70% activity after ten repeated batch reaction and could be stored up to 60 days at 4 °C with minor loss in activity. Chitosan hydrolysis using different substrates were studied using immobilized chitosanase in batch conditions. Continuous selective production of chitooligosaccharides (dimer to hexamer) by changing the temperature was achieved by PANNFM-chitosanase.

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1. Introduction
Chitosanases are group of hydrolytic enzymes which act on chitosan and can be of great industrial application due to enormous availability of chitin and chitosan in nature (Somashekar and Joseph, 1996). Chitosan is deacetylated chitin; most abundant polymer after cellulose and due to its biological properties and biocompatibility (Kim, 2010) has great potential to be applied in different areas like agricultural, industrial, biomedical, etc. Poor solubility of chitosan makes it difficult to be used at large scale. Interestingly, low molecular weight chitosan (LMWC) and chitooligosaccharides (COS) are readily soluble in water due to free amino group in β-glucosamine (Jeon et al., 2000) and show excellent biological activities (Kim and Rajapakse, 2005). Monomers of chitosan (β-glucosamine and N-acetyl β-glucosamine) has biomedical applications like arthritis treatment, dentistry, wound healing, etc. and has been studied as food supplement (Kajimoto et al., 1998). Chitosan can be depolymerized either by chemically or enzymatically. However, chemical method is avoided because of low yield, toxicity, pollution, high cost and non fitness for human consumption. Use of chitosan degrading enzyme is limited at industrial scale due to its high cost and limited availability (Kim and Rajapakse, 2005). Chitosanase enzymes have been found in large number of microbes including bacteria and fungi (Somashekar and Joseph, 1996) which can be used in production of COS, but most of these enzymes have low substrate specificity and enzyme is inducible in nature (Shimosaka et al., 1995). In order to find a novel microbial chitosanase, we screened microbes from soil sample rich in fish waste and isolated partially purified enzyme was immobilized on electrospun polyacrylonitrile nanofibrous membranes (PANNFM). Enzyme immobilization improves reusability and has other advantages like scale up, ease in recycling, continuous operation and product purification. Performance of immobilized enzyme generally depends on choice of matrix and method of immobilization. One dimensional nanofibers have extremely high surface area to volume ratio and excellently interconnected pore structure. The interconnectivity of electrospun supports circumvent the mass transfer limitations and have been used as immobilization matrix for a number of enzymes (Wang et al., 2009). NFM’s from natural polymers are generally less stable chemically and mechanically than those from synthetic polymers. PAN is a polymer with good stability and mechanical properties (Kim et al., 2005). Derivatives of PAN have also been used for enzyme immobilization with an aim to introduce functional groups into the polymer backbone due to the inertness and hydrophobicity of acrylonitrile monomer (Ye et al., 2006). In this study, PANNFM was used for immobilization of chitosanase after activation of surface by amidination reaction and were studied for residual activity, reusability, optimum pH and temperature. PANNFM-chitosanase was used for batch hydrolysis of different chitosan substrates and selective production of glucosamine and COS was achieved.