Studies on Polypropylene Hollow Fiber Membrane for Immobilization of *Arthobacter sp.* (ABL) Lipase

by

KAVITA ABROL

Centre for Polymer Science and Engineering

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① Polypropylene fibers ② Immobilization of Catalyst

DEDICATED

TO

MY PARENTS

AND

HUSBAND

CERTIFICATE

This is to certify that the thesis entitled **"Studies on Polypropylene Hollow Fiber Membrane for Immobilization of *Artobacter sp.* (ABL) Lipase"** submitted by **Ms. Kavita Abrol** to the **Indian Institute of Technology, Delhi** for the award of degree of Doctor of Philosophy is a record of bonafide research work carried out by her. Ms. Kavita Abrol has worked under our guidance and supervision and has fulfilled the requirements for the submission of this thesis.

The results contained in this thesis are original and have not been submitted in partial or full, to any other university or institute for the award of any degree (or) diploma.

(Dr.G.N.Qazi)

Director
Regional Research Laboratory,
Canal Road,
Jammu -108001

(Dr. Anup K. Ghosh)

Professor and Head of Department
Centre for Polymer Science & Engineering,
Indian Institute of Technology, Delhi
Hauz Khas, New Delhi-110016

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Kavita Abrol

(Kavita Abrol)

Abstract

Hollow fiber membrane reactors offer advantage to integrate catalytic conversion, product separation and catalyst recovery into a single separation process with respect to conventional reactors. Various membrane materials from hydrophilic to hydrophobic such as nylon, polyacrylonitrile, polyethylene, polypropylene, cellulose and polysulphone fibers have been reported in the literature. Polypropylene (PP) hollow fiber membrane is a chemically inert and stable membrane with high potential for enzyme immobilization. The surface properties of polypropylene can be modified by radiation induced grafting. Lipases, known as triacylglycerol ester hydrolase play an important role in biotechnology. Immobilization of lipases improves the economics of the process and its thermo-and chemical stability. Numerous efforts have focused on the preparation of lipase in immobilized forms involving a variety of immobilization methods and support materials. This work investigates the surface modification of polypropylene membrane, which is in hollow fiber form as the matrix for lipases immobilization. An anion–exchange porous polypropylene membrane was developed by gamma irradiation of glycidyl methacrylate (GMA) with varying degree of grafting and subsequent conversion of the produced epoxy group into diethyl amino group acting as a spacer. *Arthobacter* lipase (ABL), isolated from *Arthobacter sp.* was bound to the ionizable polymer chains grafted onto the pore surface. After crosslinking of the enzyme with glutaraldehyde, ABL was assayed for activity, pH and temperature stability.

In the first phase the grafting and amination conditions for polypropylene hollow fiber membrane by simultaneous gamma irradiation were optimized by variation of radiation dose, GMA concentration, solvent composition and inhibitor type. Grafting experiments indicated that the degree of grafting is a linear function of radiation dose. However, the radiation dose was varied from 0.54 KGy/h to 1.08 KGy/h because radiation doses higher than 1.08 KGy/h increased the viscosity of the reaction mixture and also deteriorated the mechanical strength of the fibers due to increased crosslinking of PP chains. Degree of grafting increased with increase in the GMA concentration initially but saturation was observed at higher concentration due to formation of homopolymer. The analysis of solvent composition inferred that addition of water to methanol as a cosolvent accelerated grafting of GMA on polypropylene hollow fiber membrane. Maximum grafting was found to occur in 35% methanol concentration. The degree of grafting was found to be independent of pore size and inner diameter. However, DEA percentage conversion was found to be higher for hollow fiber with smaller pore size but larger inner diameter i.e. higher specific area.

In the second phase the modified polypropylene membranes were characterized by DSC, TGA, X-ray, contact angle measurement, SEM and AFM to examine the effect of grafting and amine treatment on thermal, crystalline an surface properties. Thermal and crystallinty studies indicated that grafting ha taken place primarily in the amorphous regions of the PP membrane. AFM studies revealed that grafting with GMA increases the surface roughness of th

PP fiber. There was no significant change observed in the surface topography on activation of the grafted surface with amine, while the immobilization of lipase on the membranes surface had relevant effect on morphology, which represented a rougher profile. SEM micrographs indicated that the pore size decreased upon grafting. The study of contact angle using dynamic measurement method had shown that grafting with GMA and subsequent amine treatment significantly improved the wettability of PP fibers. In order to investigate the effect of grafting and amine treatment on mechanical properties of polypropylene hollow fiber membranes were assessed by tensile testing method. It was concluded that the tensile properties were not affected significantly by the gamma irradiation with the selected radiation doses and subsequent amine treatment. A marginal decrease in the tensile strength was correlated to the decrease in crystallinity due to dilution of the crystalline structure by amorphous graft chains.

Finally, in the third phase the modified membranes with different pore size and inner diameter having varying degrees of grafting and diethyl amine conversion were used for immobilization of ABL lipase. An attempt to correlate immobilization with DEA density revealed that a high degree of grafting and diethylamine conversion is required for efficient binding. It was also observed that the activity of ABL immobilized on DEA-EA membranes increased marginally with increase in pore size due to increased mass transfer of the substrate to the immobilized enzyme. As a matrix polypropylene porous membrane, containing ionizable polymer chains grafted onto the pore surface

proved to be effective for immobilization of lipase enzymes. The enzyme was crosslinked to prevent leaching induced by changes such as pH and temperature of the surrounding solution. Thermal stability studies showed that the immobilized lipase retained its activity at levels of 21% at temperature as high as 80^0C during a 120 min incubation period. However, the native enzyme loses its activity after 50^0C. pH studies revealed that the optimum pH for the immobilized enzyme remained unchanged. Furthermore, the pH profiles of the immobilized lipases were broader than that of the free enzyme that loses activity at pH 4.0, which means that the immobilization methods stabilized the enzyme activity over a wider pH range.

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