Galacto-oligosaccharides (GOS) contain three to ten molecules of glucose and galactose connected through glycosidic bonds. They are widely applied as non-digestible food ingredients in the nutritional market because of their beneficial health effects on stimulating Bifidobacteria in the lower part of the human intestine. GOS can be produced from whey permeate or lactose by enzymatic transgalactosidation using β-galactosidase. However, the same lactase enzyme also catalyzes the hydrolysis of lactose to glucose and galactose, which not only reduces GOS yield but also inhibits the enzyme reaction. It is thus desirable to remove these monosaccharides from the reaction medium to promote GOS production. The goal of this project was to evaluate the feasibility of enzyme immobilization on commercial nanofiltration membrane and its application on production and purification of GOS from whey lactose in a cross-flow system. In this work, Aspergillus oryzae lactase enzymes were immobilized on commercial NF membrane sheets by using polyethyleimine (PEI). The principle of our immobilization method will base on the charge interactions on protein molecules and surface charges of the support membrane. After that, these immobilized membranes were studied in the cross flow system for simultaneous GOS production and separation. Our results in a dead-end system showed that the glucose and galactose concentrations in the final GOS product were reduced approximately to 50% and 65%, respectively. Also, % GOS from the simultaneous production and separation were increased by 15% as compared with those from the conventional method. Clearly, the %GOS and GOS yield from the enzyme reaction have been greatly increased by the removal of the undesired byproducts (glucose and galactose). Therefore, this process can reduce the cost for GOS production from whey lactose, a byproduct from the dairy industry, by improving product yield and reducing undesirable sugars in the final GOS product.